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VOL. 21, SEC. C. NOVEMBER, 1943 NUMBER 11

STARCH CONTENT OF WESTERN CANADIAN WHEAT

II. ITS ESTIMATION FROM PROTEIN CONTENT, AND SOME ESTIMATED DATA[1]

BY J. ANSEL ANDERSON[2] AND WILLIAM J. EVA[3]

Abstract

Data for 180 samples representing 20 carlots of each of Grades 1 Northern, 3 Northern, and No. 5 wheat, inspected at Winnipeg, Edmonton, and Calgary, were used in a study of the relations between starch and protein content. It was demonstrated that all data for Grades 1 and 3 Northern could be legitimately pooled. The resulting correlation coefficient was $-.918$, and the prediction equation was: starch $= 68.0 - 1.12 \times$ protein (± 0.76). The data for No. 5 wheat were heterogeneous, and yielded correlation coefficients of the order of $-.7$ which were too low for prediction purposes. By means of the above equation it is estimated that for the past 16 crops the average starch content of Western Canadian wheat has varied between 51.1 and 55.1%, with a mean value of 52.7% (13.5% moisture basis). Starch maps are shown representing the average for the 12 year period 1927 to 1938, and the crops of the two years, 1941 and 1942.

The increasing use of wheat by the fermentation industries in Canada and the United States has created an interest in the starch content of Western Canadian wheat. Earlier data published by Herd and Kent-Jones (5), Hopkins and Graham (7), and Hopkins (6) have been recently supplemented by a study made in this laboratory (4). In addition, while this paper was in preparation, we received from McCalla and Corns the manuscript of a paper, since published (8), dealing with starch and protein contents, and the relation between them, for samples representing several varieties of wheat and barley grown in a number of different localities. In spite of these recent additions, the data on the starch content of Western Canadian wheat are still meagre by comparison with data available on protein content. The latter have been collected in this laboratory since 1927, and a summary of 104,000 data representing the crops of the 12 years 1927 to 1938 have recently been published (2). As the starch and protein contents of wheat are inversely related it appeared that information on the former might be usefully supplemented by suitable interpretation of summarized protein data.

As a first step it was necessary to derive an equation for predicting starch content from protein content. The study parallels that made by McCalla and Corns, but was made with 180 samples taken from carlot shipments of

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different grades of wheat. With the aid of the equation, the range over which the average starch content of Western Canadian wheat may be expected to fluctuate is shown by listing estimated starch contents for the crops of the past 16 years, and the variations in starch content that occur from district to district are illustrated by means of starch maps.

Materials and Methods

For purposes of grading, a sample is taken by the Inspection Branch of the Board of Grain Commissioners from each carlot of grain entering commercial channels. The principal inspection offices are Winnipeg, Edmonton, and Calgary; and 20 samples of No. 1 Northern, 20 samples of No. 3 Northern, and 20 samples of No. 5 wheat were therefore collected from each of these three inspection offices for the present study. In making the collections, attention was paid to protein content so that the samples for each grade and office might cover with reasonable uniformity as wide a range of protein content as possible.

All samples were cleaned free from dockage and two subsamples, one for the determination of starch and protein, and the other for the determination of 1000-kernel weight, were prepared with a Boerner sampler. The subsample for analysis was ground in a burr mill and then reduced to a fine powder by ball-milling for 24 hr. Starch was determined by the Clendenning (3) method, and protein by the Kjeldahl-Gunning-Arnold procedure. Duplicate subsamples of the ground material were analysed in random order for both starch and protein. Weight per 1000 kernels was determined by counting the number of kernels in a 30 gm. sample of the whole wheat. All results are reported on a 13.5% moisture basis.

Results and Discussion

Mean data for the protein content, starch content, and 1000-kernel weight, of each grade at each inspection office, are listed in the upper section of Table I. Edmonton values are characteristically higher for starch and lower for protein than those for the other two offices. The mean values over all offices show a decrease in protein content, and an increase in starch content and 1000-kernel weight, with decreasing grade. These orders are held moderately well with respect to protein and starch content at each office, but considerable variations occur in the rank orders with respect to kernel weight.

The ranges for each property are shown in the lower section of the table. For purposes of studying the relation between two variables, and particularly if it is desirable to establish the regression coefficient with precision, it is

TABLE I

MEAN VALUES AND RANGES FOR PROTEIN, STARCH, AND 1000-KERNEL WEIGHT OF WHEAT

Grade	Property	Inspection office			All offices
		Winnipeg	Edmonton	Calgary	
Mean values					
1 Northern	Protein, %	14.3	12.0	14.2	13.5
	Starch, %	51.9	54.5	52.0	52.8
	1000-kernel wt., gm.	27.2	31.9	29.2	29.4
3 Northern	Protein, %	14.1	12.1	12.5	12.9
	Starch, %	52.2	54.6	53.9	53.6
	1000-kernel wt., gm.	24.7	30.9	33.1	29.6
No. 5	Protein, %	12.5	11.3	12.4	12.1
	Starch, %	53.2	54.4	54.0	53.9
	1000-kernel wt., gm.	28.0	30.6	32.8	30.5
Ranges					
1 Northern	Protein, %	12.3–17.4	10.6–14.4	12.4–15.8	10.6–17.4
	Starch, %	48.3–54.4	51.2–55.5	49.4–53.8	48.3–55.5
	1000-kernel wt., gm.	22.0–30.1	26.1–34.3	24.6–33.4	22.0–34.3
3 Northern	Protein, %	11.8–17.0	9.0–14.0	9.7–16.4	9.0–17.0
	Starch, %	48.6–54.9	51.9–57.0	49.1–57.9	48.6–57.9
	1000-kernel wt., gm.	20.4–30.7	25.2–34.7	22.7–38.8	20.4–38.8
No. 5	Protein, %	11.0–14.1	9.7–12.8	11.0–14.2	9.7–14.2
	Starch, %	50.4–56.4	52.6–56.5	52.0–56.0	50.4–56.5
	1000-kernel wt., gm.	17.2–37.8	28.0–34.1	27.2–36.7	17.2–37.8

advantageous to have a series of samples covering a wide range of values for each variable. Considered from this viewpoint, the samples of 3 Northern were satisfactory, those for 1 Northern were fairly satisfactory, but those for No. 5 wheat covered rather a narrow range. Nevertheless, the samples of each grade from each office represent as wide a range as could be conveniently collected, and the narrower ranges for No. 5 wheat indicate that carlots of this grade did not vary widely in starch and protein content in 1942.

The correlation coefficients for starch and protein content are given in Table II. It is interesting to note that the magnitude of these coefficients follows closely the ranges for starch and protein content shown in Table I. All correlation coefficients attain the 1% level of statistical significance. Those for Grades 1 and 3 Northern lie between $-.80$ and $-.93$, except for 1 Northern at Calgary which yielded a coefficient of only $-.73$. Coefficients for No. 5 wheat were lower than those for the higher grades, except at Calgary where that for 1 Northern was lowest.

TABLE II

CORRELATION AND REGRESSION COEFFICIENTS FOR STARCH AND PROTEIN CONTENT OF WHEAT

Grade	Inspection office			All offices
	Winnipeg	Edmonton	Calgary	
Correlation coefficient				
1 Northern	−.886	−.806	−.734	−.893
3 Northern	−.922	−.885	−.931	−.936
No. 5	−.595	−.775	−.740	−.703
Regression coefficient				
1 Northern	−1.26	−1.01	−0.88	−1.10
3 Northern	−1.05	−1.02	−1.23	−1.11
No. 5	−0.82	−0.86	−0.77	−0.81

The regression coefficients for starch and protein content are given in Table II. An analysis of residual variance failed to demonstrate significant difference between regression coefficients, but showed differences between centroids. The regression lines and centroids for Grades 1 and 3 Northern are shown in Figs. 1A and 1B. Inspection suggested that these did not differ

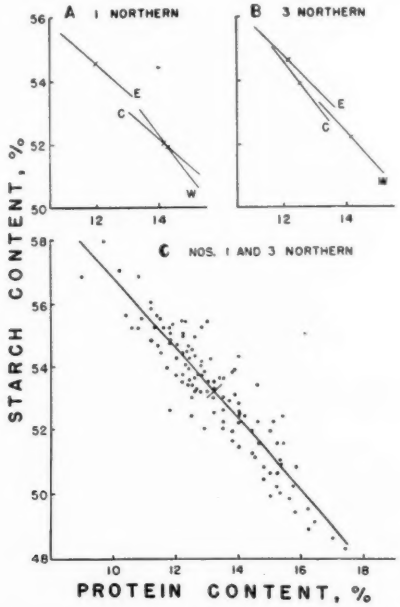

FIG. 1. *1A and 1B, regression lines for samples of 1 Northern and 3 Northern, respectively, for samples from the Edmonton (E), Calgary (C), and Winnipeg (W) Inspection offices. 1C, scatter diagram for all samples of both grades.*

significantly, and this was confirmed by separate analysis of the data for these two grades. Accordingly, it appeared permissible to pool the results for 1 and 3 Northern. The resulting scatter diagram is shown in Fig. 1C; it represents a correlation of $-.918$ and a regression coefficient of -1.12.

The scatter diagrams for No. 5 wheat are given in Fig. 2. They show that the line for Calgary lies considerably above those for the other two offices; and this was confirmed by an analysis that showed that a significant difference existed between centroids. This heterogeneity, together with the low correlation coefficients (Table II), indicated that there was little hope of predicting the starch content of No. 5 wheat. Confirmation of this opinion was obtained

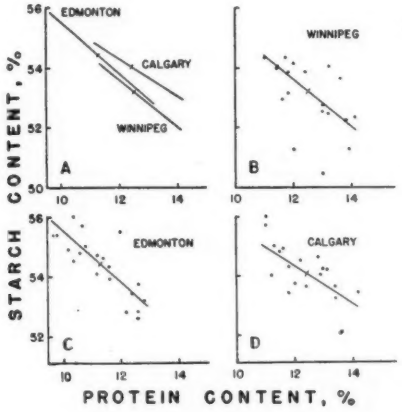

FIG. 2. *2A, regression lines for samples of No. 5 wheat from three Inspection offices. 2B, 2C, and 2D, scatter diagrams for samples from each office.*

when the relations with 1000-kernel weight were examined and further heterogeneity was encountered. This situation is not surprising in view of the wide differences that occur in the quality characteristics of carlots of wheat that are graded No. 5. Under the circumstances, it does not seem useful to give details of the additional studies made with data for No. 5 wheat.

An examination of the relations between 1000-kernel weight (k), protein (p), and starch (s) was undertaken with the pooled data for Grades 1 and 3 Northern. The pertinent simple and partial correlation coefficients follow:—

$$r_{sp} = -.918 \qquad r_{sk} = .684$$
$$r_{sp.k} = -.843 \qquad r_{sk.p} = .035$$

The interpretation of these statistics in terms of the proportion of all environmental factors common to the control of each pair of properties presents interesting problems in deduction and exposition. As the matter is not germane to the present investigation, which seeks only a method for predicting starch content, it need not be examined. It is necessary to note only that the partial correlation between starch and kernel weight, independent of

protein content, is extremely low. This indicates that no advantage can be gained by attempting to predict starch content from both protein and kernel weight. The multiple correlation ($R_{s.pk}$) is .920 and analysis showed that this was not significantly greater than the simple correlation, $-.918$. These results are in accord with those of McCalla and Corns (8).

These authors obtained an increase in the correlation coefficient by adding bushel weight as a second independent variable. A corresponding examination was therefore made with our data. For this purpose it was possible to obtain bushel weights for all samples of 1 and 3 Northern except those for No. 1 Northern from Edmonton. The simple and partial correlations for the 100 remaining samples follow (s = starch, p = protein, b = bushel weight):—

$$r_{sp} = -.915 \qquad r_{sb} = .416$$
$$r_{sp.b} = -.897 \qquad r_{sb.p} = .098$$

The statistics duplicate those for kernel weight and require no further discussion. With our data, no advantage can be obtained in predicting starch by including both protein content and bushel weight as independent variables.

The prediction equation for Grades 1 and 3 Northern—and it may be assumed that it will apply equally well to Grades 1 Hard and 2 Northern—is as follows:

$$\text{Starch} = 68.0 - 1.12 \times \text{protein}.$$

The error of estimation is quite high, namely, ± 0.76. Inspection of the scatter diagram in Fig. 1C will show that a number of the points lie considerably above or below the regression line. Some of this scatter doubtless results from varietal differences between samples; for McCalla and Corns have shown that the regression lines for different varieties do not coincide.

In the present study the regression of starch on protein content was found to be -1.12 ± 0.044. McCalla and Corns obtained a value of -1.25. It should be noted, however, that they used the Hopkins modification of the Mannich-Lenz method whereas the Clendenning modification was used in our investigation. Under these conditions, and particularly when the partial correlation and regression coefficients are taken into account, the agreement between the two studies appears satisfactory. In any event, since the direct determination of starch is comparative rather than absolute with methods now available, it follows that the prediction of starch from protein data must be comparative also. The writers believe that the prediction equation given above can be used satisfactorily for providing a general picture of the variations in starch content that occur from district to district and from year to year in the higher grades of Western Canadian hard red spring wheat. In this connection it should be borne in mind that though the error of estimation is quite high for samples representing single carlots of grain, it may be expected to be low for data representing large numbers of carlot shipments.

Estimated Starch Contents

By using the prediction equation given in the preceding section and the protein data that have been accumulated in this laboratory during the past 16 years, it is possible to obtain a fairly comprehensive picture of the variations that occur in the starch content of Western Canadian wheat. Although the protein data are readily available (1, 2), and the task of estimating starch contents from them is simple, it appeared useful to publish some summaries of estimated starch data in this paper.

In Table III the estimated average starch contents for crops of each of the past 16 years are given for Western Canada as a whole, and for each of the three Prairie Provinces. The starch content of the whole crop has varied between 51.1 and 55.1%, with a mean value for the 16 years of 52.7% (13.5% moisture basis). In 12 of the 16 years the starch content fell within ±1.0% of the mean; in two of the driest years it was very low, and in two of the wettest years it was very high. Among the provinces, Saskatchewan tends to produce a crop that is about 0.5% lower in starch content than crops from the other two provinces.

TABLE III

ESTIMATED STARCH CONTENTS (%, 13.5% MOISTURE BASIS) OF WESTERN CANADIAN HARD RED SPRING WHEAT FOR THE YEARS 1927 TO 1942

Year	Western Canada	Province		
		Alberta	Saskatchewan	Manitoba
1927	55.1	55.1	55.1	55.6
1928	54.2	54.4	54.0	54.4
1929	53.0	53.0	52.9	54.2
1930	53.2	53.0	53.1	54.8
1931	53.0	53.7	52.4	52.0
1932	52.4	52.9	52.1	52.5
1933	52.7	53.1	52.3	52.5
1934	52.5	53.1	52.0	52.5
1935	52.3	52.5	52.0	52.8
1936	51.5	52.7	51.1	50.8
1937	52.2	52.4	51.3	52.3
1938	52.3	52.7	51.9	52.8
1939	52.2	52.7	52.0	51.9
1940	52.2	53.1	52.0	51.8
1941	51.1	51.5	50.5	51.8
1942	53.7	54.1	53.6	53.4
Mean	52.7	53.1	52.4	52.9

Fig. 3A shows the starch map for the 12 year period 1927 to 1938; it is based on the data used in the corresponding protein map (2). While it would have been preferable to bring the map up to date by including data for the past four years, the task is a very substantial one, and it seemed certain that only a few minor changes would occur in the map. Inspection will show that the principal high starch areas lie north and west of Edmonton, in northeastern Saskatchewan, and in the irrigation districts of southern Alberta.

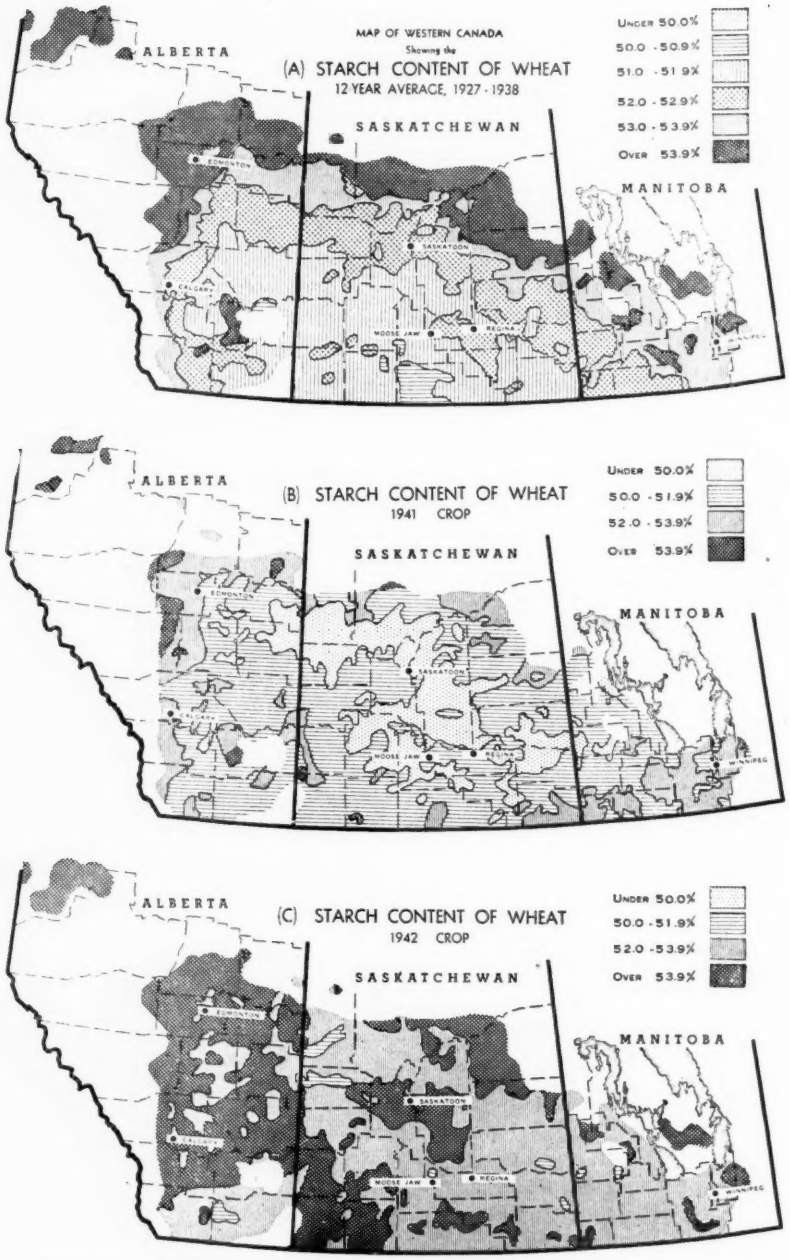

FIG. 3. *Maps of Western Canada showing the average starch content of hard red spring wheat for: A, the 12 crops, 1927 to 1938; B, the 1941 crop; and C, the 1942 crop.*

Figs. 3B and 3C show the starch maps for the years 1941 and 1942. They are interesting because they represent the bulk of the wheat now in storage in Canada, and because they illustrate the enormous differences which can occur between the starch maps for different years. It is hardly necessary to add that the 1941 season was very dry and produced a crop of high protein and low starch content, whereas the 1942 season was wet and produced a crop of low protein and high starch content.

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THE STORAGE AND ARTIFICIAL GERMINATION OF FOREST TREE POLLENS[1]

By L. P. V. Johnson[2]

Abstract

The physical and nutritional requirements for pollen germination in several forest tree species were studied by using a variety of nutrient combinations in agar, silica gel, and distilled water media. It was found that 10% sucrose in 0.75% agar was the best of these media for *Pinus* pollen, while 1.13% bean pod agar was best for *Picea*.

Results from studies on light and dark incubation of *Pinus*, *Picea*, and *Quercus* pollens showed no significant difference in percentage germination, but indicated a stimulation of pollen tube growth by light.

Pollens of several species were stored under different conditions of relative humidity (graded series, 0 to 75%), temperature (2° C. and room temperature), and light (light and dark). Data on germination after storage for one year showed: that pollen longevity of all viable species was greatly favoured by low temperature and darkness; that the best relative humidity was: for *Pinus*, 15 to 35% at room temperature and 50 to 75% at 2° C., for *Picea* 15% at room temperature and 10 to 75% at 2° C., and for *Quercus* 25 to 35% at 2° C. (no germination at room temperature).

Highest percentage germination after storage for one year ranged from 95 to 99% for *Pinus* species, 58 to 63% for *Picea* species, and 46% for a *Quercus* species (2° C., dark, in each case).

In all species successfully stored, pollen germination was higher after 12 months' storage than after eight months' storage.

Introduction

In connection with an investigation on the breeding of forest trees (3) a practical need has arisen for convenient and efficient methods of storing and testing the various kinds of pollen used in hybridization work.

Recently there has been a number of valuable contributions in this field, but existing information is still decidedly inadequate. The storage and germination requirements of *Pinus Strobus* and *P. resinosa* have been studied by Duffield and Snow (2), and the germination of *P. ponderosa* by Righter (5); but for many important species of the genus these requirements remain largely unknown. The work of Dengler and Scamoni (1) provided valuable new information on the artificial germination of pollen of several genera, but did not include studies on storage. The present work was undertaken in an effort to fill some of the gaps in the existing knowledge of storage and germination requirements of forest tree pollens, especially those deficiencies that were felt in the author's work on hybridization of forest trees.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1156.

[2] *Forest Geneticist, National Research Laboratories.*

Materials and General Methods

The pollens used in the various tests were collected from the following species:

Pinus Strobus L. (white pine)
resinosa Ait. (red pine)
sylvestris L. (Scotch pine)
Banksiana Lamb. (jack pine)
attenuata × *P. radiata*, F_1
Picea Abies (L.) Karst. (Norway spruce)
glauca (Moench) Voss No. 1 (white spruce)
glauca (Moench) Voss No. 2 (white spruce)
Quercus coccinea Muenchh. (scarlet oak)
Betula lutea Michx. (yellow birch)
Larix siberica Ledeb. (Siberian larch)
Kaempferi Sarg. (Japanese larch)
Populus tremuloides Michx. (quaking aspen)
deltoides Marsh. (cottonwood)

Pine and spruce pollens proved to have the greatest longevity in storage and, as a result, provided most of the data, not only on storage requirements, but on germination requirements as well.

Accurately controlled incubation conditions were provided by a bunker-type germinator in which air was drawn through the heating and cooling units and then forced around on all sides of hermetically sealed germination chambers. The same chamber was used for all experiments, except that in which duplicate germinations were carried out simultaneously in light and dark. The light was provided by two 20-watt fluorescent tubes, one recessed on either side of the chamber. All chambers were provided with a water tray which maintained humidity approaching the saturation point. Temperature was maintained at 25° C. (77° F.).

Incubation periods varied with species and media. In distilled water, all species were incubated for 48 hr. On the "solid" media, spruce species were incubated for 72 hr.; oak, 120 hr.; pine, 96 to 144 hr.; and all non-germinating species (birch, larch, etc.), 144 hr.

In microscopic observation a 6× ocular was used, combined with a 16 mm. objective for spruce species and with a 12.6 mm. objective for all other species. Germ-tube measurements were made with a grid-type ocular micrometer.

Germination counts were for the most part based on the random selection of a single field containing 30 or more pollen grains that could be definitely distinguished as germinable or non-germinable. In the final experiment, however, 50 grains were always counted, which often entailed shifting the slide to disclose additional grains adjacent to a field of less than 50 grains.

Experimental Results

The experiments reported herein involved the following steps: 1. Selection of superior germination media, based upon (*a*) tests of three substrata (agar, silica gel, and distilled water) to each of which a graded nutrient series was added, and (*b*) experiments using a similar nutrient series on selected substrata and also a number of standard agar media. 2. Studies on the effect of light and dark incubation on percentage germination and growth rate of pollen tube. 3. Effect on pollen longevity of storage for one year under different conditions of humidity, light, and temperature.

Selection of Germination Media

Previous workers on the artificial germination of pollen, using a wide variety of media, have shown that pollen of certain species have highly specific requirements. It was therefore thought wise to determine the particular germination requirements of the pollens under study prior to investigating the relation between storage conditions and longevity.

Preliminary tests of fresh (1940) poplar, birch, and pine pollens on 0.75, 1.0, 1.5, and 2.0% agar, each nutrified with a series of sucrose concentrations, demonstrated that 0.75% agar tends to give higher germination than the stronger agars. This is in agreement with results of Traub and O'Rork (6) on papaya pollen.

Tests of Agar, Silica Gel, and Distilled Water as Substrata

Experiments were conducted on 0.75% agar, silica gel, and distilled water, used alone and with the addition of various combinations of sucrose and yeast extract (see Table I).

The agar media were made up by adding the sugar and yeast to autoclaved flasks after they had cooled to 50° C. Difco Bacto agar and Difco Bacto yeast extract were used. Each agar medium was then poured about $\frac{1}{8}$ in. thick on a porcelain tray, cut when firm into pieces $\frac{7}{16}$ in. square, and placed in sterilized Petri dishes according to a randomized plan. The bottom of each dish was marked with a china marking pencil into $\frac{9}{16}$ in. squares, four units to a side. A numeral–letter system of labelling rows and columns (Latin square) provided designation for each individual square. Two pieces of each medium were placed in each dish, and after germination duplicate counts were made on each piece. Pollen was applied by blowing the grains into the air over the dish from opposite directions. To prevent contamination each kind of pollen was applied in a different room, only one kind of pollen being applied to any one dish.

The silica gel media were prepared as follows: the gel (silica acid) was made by treating sodium silicate with hydrochloric acid, the sodium chloride residue leached out in tap water (tested for complete leaching with silver nitrate), a piece of the gel placed in a Petri dish and dried under unglazed porcelain

covers until beginning to crack, then soaked overnight in appropriate nutrient solution. The gel was then cut into $\frac{7}{16}$ in. pieces and used in the manner described for agar. The pH of the gel was approximately 7.

The distilled water media were prepared by adding the necessary nutrients and placing the solution as hanging drops in holes cut in the paraffined bottom of a Petri dish according to the method of Righter (5). Two replicate drops of each medium were used, pollen being blown over the dishes as described above. Only a single germination count was made on each drop.

The pollen material studied consisted of seven species of pine, two of poplar, and one each of spruce, birch, and ash collected in the spring of 1940 and stored under three different levels of relative humidity for approximately nine months. Only pine and spruce pollens germinated; the highly summarized results for two representative pine species and one spruce species are given in Tables I and II.

TABLE I

AVERAGE PERCENTAGE GERMINATION ON THREE SUBSTRATA (NUTRIFIED) TAKEN OVER TWO PINE AND ONE SPRUCE SPECIES

Nutrient added	Substratum			Nutrient means
	Silica gel[1]	0.75% Agar[1]	Water[2]	
None	1.8	18.6	18.4	12.9
2.5% sucrose	0.5	19.9	12.9	11.1
2.5% sucrose + 0.2% yeast extract	1.8	18.1	9.3	9.7
5% sucrose	1.8	27.0	13.3	14.0
5% sucrose + 0.2% yeast extract	0.4	14.7	13.2	9.4
10% sucrose	0.1	31.4	11.8	14.4
10% sucrose + 0.2% yeast extract	0.2	22.7	7.5	10.1
0.2% yeast extract	1.0	15.4	8.7	8.4
Average	1.0	21.0	11.9	—

[1] *Averages based on 36 samples of about 40 pollen grains each.*
[2] *Averages based on 18 samples of about 40 pollen grains each.*

TABLE II

AVERAGE PERCENTAGE GERMINATION OF EACH SPECIES ON EACH SUBSTRATUM TAKEN OVER ALL APPLIED NUTRIENTS

Substratum	Kind of pollen		
	White pine	Korean pine	Norway spruce
Silica gel[1]	2.6	0.3	0.0
0.75% agar[1]	47.4	9.0	6.5
Water[2]	21.0	13.0	1.7

[1] *Averages based on 96 samples of about 40 grains each.*
[2] *Averages based on 48 samples of about 40 grains each.*

The data presented in Table I show: that, as an average over all species of pollen, agar and distilled water, without nutrient, gave almost identical percentages of germination, while silica gel gave very low germination; and that nutrients when added to silica gel had little or no effect, when added to agar had certain definite beneficial effects, and when added to distilled water tended to have a deleterious effect.

The results based on the reaction of the individual pollen species to the various substrata (Table II) show that silica gel was decidedly inferior for all species, agar superior for white pine and Norway spruce, and distilled water superior for Korean pine. The inferiority of silica gel was probably due to the fact that it was considerably drier than the agar used.

It was concluded from the above experiment that silica gel was definitely inferior to the other two substrata, and could be dropped from further consideration in the study, and that both agar and distilled water should be tested further.

Tests of Various Nutrients in Agar and Distilled Water

A second experiment, along similar lines to that described above, was designed to test further the nutrient requirements for germination in forest tree pollens, using 0.75% agar and distilled water as substrata. The agar and water media were prepared, and the arrangement of randomized squares, application of pollen, germination counts, etc. carried out in the same way as before. The silica gel series, however, was replaced by eight standard agar media, each in a concentration calculated to give a 0.75% agar substratum (see Table IV).

The pollen material was collected in May, 1941, from red, jack, and (*Pinus attenuata* × *P. radiata*) F_1 pines, white and Norway spruces, Japanese and Siberian larches, scarlet oak, and yellow birch. All material was stored for several days over anhydrous calcium chloride prior to testing.

The larch and birch[1] pollens failed to germinate. Pollen grains of both larch species absorbed much water, became greatly distended and were frequently ruptured. It was found impossible to make germination counts on the oak pollen owing to a rapid growth of mould that quickly covered the media.

[1] *Subsequent experiments on B. lutea and B. papyrifera, using 0.75, 1.0, 1.5, and 2.0% agar and distilled water each nutrified with 0, 10, 20, and 30% sucrose, show that fresh pollen of either species will give about 40% germination on the 0.75% agar and 20% sucrose medium. B. papyrifera also germinated fairly well on all the higher sugar concentrations in all substrata, while B. lutea was exacting in requiring the medium stated. After one month's storage at 5, 15, 25, 35% relative humidity at room temperature (dark), tests on both species indicated that 25% humidity was optimal, B. lutea (again very exacting) averaging 5% and B. papyrifera 2% germination.*

The results of tests on the two spruce species and on two representative pine species are given in highly summarized form in Tables III and IV.

TABLE III

AVERAGE PERCENTAGE GERMINATION ON AGAR AND WATER

Nutrient added	0.75% Agar[1]				Distilled water[2]			
	Jack pine	Red pine	Av. for pines	Norway spruce	Jack pine	Red pine	Av. for pines	Norway spruce
None	17.3	6.5	11.9	3.8	4.9	9.0	6.9	6.9
2.5% sucrose	9.4	21.3	15.3	25.9	6.7	38.1	22.4	7.5
2.5% sucrose + 0.3% yeast extract	17.8	22.7	20.2	11.8	6.5	11.3	8.9	1.5
5% sucrose	7.5	17.5	12.5	22.7	4.5	37.1	20.8	11.0
5% sucrose + 0.3% yeast extract	29.4	25.4	27.4	0.0	9.2	13.3	11.2	0.6
10% sucrose	50.0	62.1	55.9	7.6	5.0	41.8	23.4	13.0
10% sucrose + 0.3% yeast extract	27.2	34.5	30.8	6.9	2.2	18.3	10.2	0.0
0.3% yeast extract	12.0	13.1	12.5	8.7	4.6	12.3	8.4	0.0

[1] *Average based on two counts of about 40 grains each on each of two replicates.*
[2] *Averages based on one count of about 40 grains on each of two replicates.*

TABLE IV

AVERAGE PERCENTAGE GERMINATION ON STANDARD AGAR MEDIA

Bacto agar media	pH	Kind of pollen				Av. for pines	Av. for spruces	Grand average
		Jack pine	Red pine	Norway spruce	White spruce			
Agar check, 0.75%	5.8	17.3*	6.5	3.8	3.5	11.9	3.6	7.8
Sabouraud's dextrose, 3.25%	5.6	10.7	11.4	31.7	19.4	11.1	25.5	18.3
Nutrient, 1.55%	7.3	39.0	16.3	0.0	1.7	27.6	0.9	14.2
Prune, 1.25%	5.5	26.6	17.8	32.3	17.4	22.1	24.9	23.5
Bean pod, 1.13%	5.8	22.0	10.2	31.8	26.8	16.1	29.3	22.7
Malt, 2.25%	5.5	8.8	8.2	25.5	10.5	8.5	17.9	13.2
Potato dextrose, 2.0%	5.6	35.6	11.1	27.2	18.9	23.3	23.1	23.4
Wort, 2.53%	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Corn meal, 0.95%	6.0	7.6	5.5	24.1	13.3	6.6	18.7	12.6

* *All averages based on two counts of about 40 grains each on each of two replicates.*

The results presented in Table III clearly demonstrate that 10% sucrose in 0.75% agar, without yeast, is optimal for pine species. The weaker concentrations of sucrose in agar, without yeast, are the most favourable for spruce pollen. Yeast extract appears to be favourable to jack pine when added to the weaker sugar concentrations, especially in agar; but it is unfavourable to red pine pollen in sugar-water media, to spruce in all nutrified media, and to all species when added to 10% sucrose in either agar or water.

Table IV gives the results from tests on various standard agar media, which indicate germinative responses to both nutritive and pH factors. The most acid of these media, wort agar (pH, 5.0), inhibited pollen germination in all

species of all genera, while the most alkaline, nutrient agar (pH, 7.3), practically inhibited germination in the spruce species, but gave good germination in pine. Jack pine responded favourably to a number of media, particularly nutrient agar and potato dextrose agar (pH, 5.6), but germination was not as high as in 10% sucrose (see Table III). None of the media were favourable for red pine. A number gave good results with spruce pollens; the best, taking the two species together, was bean pod agar (pH, 5.8), which was better for spruce pollens than any of the media dealt with in Table III.

It was concluded, therefore, that, of the media tested, 10% sucrose in 0.75% agar was best for germinating pine pollens; and that 1.13% Bacto bean pod agar was best for spruce pollens.

All subsequent germination tests were carried out in accordance with this conclusion.

Effect of Light and Dark Incubation on Germination and Growth of Pollen

The effect of light and dark on pollen germination and growth was studied by incubating duplicate samples (in Petri dishes) simultaneously in chambers differing only in that one was lighted by two 25-watt fluorescent tubes while the other was dark. The pollen used had been stored for eight months under various conditions of light, humidity, and temperature, the results given in Table V being averaged over all conditions.

TABLE V

Effect of light and dark incubation on germination and on growth of germ tube

Species	Av.[1] percentage germination		Av.[1] length of germ tube (μ)	
	Light	Dark	Light	Dark
F_1 hybrid pine	17.6	21.0	6.6	5.4
Jack pine	54.7	56.4	9.5	8.9
Korean pine	16.3	17.4	6.9	6.5
Red pine	35.6	35.4	11.0	9.0
White pine	46.7	42.6	8.6	7.7
Norway spruce	18.5	18.7	16.5	14.1
White spruce—1	9.4	10.7	10.1	10.1
White spruce—2	13.7	13.8	11.0	9.9
Scarlet oak	5.0	4.9	9.1	5.0
Av. pine spp.	34.2	34.6	8.5	7.5
Av. spruce spp.	13.9	14.4	12.5	11.4

[1] *Average based on 20 samples of about 40 pollen grains each.*

There was no statistically significant difference in germination data after incubation under light or dark conditions. There was, however, a statistically significant increase in germ tube length in samples incubated in light. These

facts may be taken to indicate that, after germination has taken place, light has a slight stimulatory effect on the rate of growth in the germ tube. However, for practical purposes, it may be concluded that it makes no difference, in the species studied, whether tests for pollen viability involve light or dark incubation.

Effect of Storage under Different Conditions of Humidity, Temperature, and Light

This experiment involved the testing of pine, spruce, and oak pollens for viability after 8- and 12-month periods of storage under various conditions of humidity, temperature, and light. Details of kinds of pollen and conditions of storage may be found in Tables V and VII.

The pollen was placed in cotton-stoppered vials and stored in 6-in. desiccators, in which relative humidity was controlled by aqueous solutions of sulphuric acid (except in some instances where desiccators containing anhydrous calcium chloride were added to the experiment). Desiccators stored in the light were so placed that they received no direct sunlight. The concentrations of sulphuric acid used to provide the different degrees of humidity were based on the data of Wilson (7).

The previous method of placing several small, square pieces of agar in a single dish was rejected for this experiment because the number of individual tests was considerably less, and because the small individual pieces tended to round up, giving a somewhat curved top surface which was considered unfavourable to uniform germination. Duffield and Snow (2), who used small round "wafers", did not experience the same difficulty because they worked with 2.0% agar, which would be much firmer and drier than the 0.75% agar used as the standard agar concentration in all experiments reported in this paper.

The media were: 10% sucrose in 0.75% agar for pine and oak; and 1.13% Bacto bean agar for spruce. Two samples from each kind of pollen in each storage condition were placed in separate Petri dishes into which 20 cc. of medium had been poured.

In the eight month test, one of the replicate dishes was incubated in light, the other in darkness (see preceding section). However, since there was no significant difference in germination under the two conditions, the results were combined.

The results of germination tests after 8 and 12 months' storage, averaged in each case on a genus basis, are given in Table VI.

TABLE VI

AVERAGE GERMINATION ON GENUS BASIS AFTER 8 AND 12 MONTHS' STORAGE UNDER DIFFERENT CONDITIONS OF HUMIDITY, TEMPERATURE, AND LIGHT

Storage conditions		Av. pine spp.[1]		Av. spruce spp.[2]		Scarlet oak[3]	
Temperature and light	Relative humidity, %	Storage period, months					
		8	12	8	12	8	12
Room temp. (light)	$CaCl_2$*	39.4	36.6	2.5	4.5	0.0	0.0
	25	49.6	57.8	3.8	7.0	0.0	0.0
	50	39.3	34.4	3.5	4.7	0.0	0.0
Room temp. (dark)	$CaCl_2$*	10.1	81.6	4.6	1.7	0.0	0.0
	0	0.0	2.8	0.0	0.0	0.0	0.0
	5	2.7	40.6	1.9	1.3	0.0	0.0
	10	5.7	74.6	2.8	3.0	0.0	0.0
	15	15.8	83.6	6.3	6.7	0.0	0.0
	25	14.9	74.4	3.3	3.7	0.0	0.0
	35	20.8	79.0	2.7	5.3	0.0	0.0
	50	28.3	44.2	1.8	2.7	0.0	0.0
	75	0.0	0.0	0.0	0.0	0.0	0.0
2° C. (dark)	0	1.7	36.2	5.4	6.8	0.0	0.0
	5	45.8	71.0	24.6	37.5	7.3	31.0
	10	51.7	81.6	24.5	45.7	9.2	34.0
	15	54.5	87.0	33.8	42.5	34.5	41.0
	25	69.0	93.4	36.0	44.7	5.5	46.0
	35	61.3	91.0	39.6	42.7	11.8	46.0
	50	81.9	94.2	37.2	48.7	26.8	11.0
	75	95.0	93.8	49.7	52.7	4.6	0.0
Necessary difference		5.8	3.0	2.3	3.6	–	–

[1] *Averages based on 10 samples of 40 to 50 pollen grains each.*
[2] *Averages based on six samples of 40 to 50 pollen grains each.*
[3] *Averages based on two samples of 40 to 50 pollen grains each.*
* *The relative humidity provided by calcium chloride was not definitely known and does not form a part of the humidity series.*

The most striking result is the much greater germination obtained from samples stored at 2° C. than from those stored at room temperature. This difference is highly significant for all genera stored under all humidity conditions from 5 to 75% inclusive (except oak, 75%, in the 12 month test).

In comparing the efficiency of storage humidities in the 2° C. (dark) and the room temperature (dark) conditions, it will be noted that at 2° C., germination in both pine and spruce tends to increase with increased humidity or to level off at the higher humidities; while at room temperature, optima are reached at 15 to 50% in pine and at 15 to 35% in spruce. Oak pollen germinated only at the lower temperature, with the optimum in the 15 to 35% humidity range.

Comparing the results after storage at room temperature in light and dark, it will be observed that, in pine, light was the more favourable after eight months, while dark gave the better results after 12 months; and that, in spruce, there was no significant difference between light and dark storage in either test.

It is interesting to note that there was considerably greater germination in pine after 12 months' storage than after eight months. This is particularly striking in the room temperature (dark) condition. A similar result is shown in Table I of Duffield and Snow (2) where both red and white pine pollen, stored under various conditions of humidity and temperature for 93- and 181-day periods, gave considerably higher germination after the longer period of storage.

Storage over calcium chloride at room temperature (light and dark) was included in the experiment since it represents a common practice for keeping pollen.

Table VII presents data on the percentage pollen germination of individual species after storage for 12 months. There is considerable specific difference in tolerance of low humidity storage at both 2° C. and room temperature.

TABLE VII

GERMINATION OF POLLEN AFTER 12 MONTHS' STORAGE UNDER DIFFERENT COMBINED CONDITIONS OF HUMIDITY, TEMPERATURE, AND LIGHT

Storage conditions		Percentage germination (av. of two counts of 50 grains each)								
Temperature and light	Relative humidity, %	Pine					Spruce			Scarlet oak
		F_1 hybrid	Jack	Korean	Red	White	Norway	White −1	White −2	
Room temp. (light)	$CaCl_2$	12	58	0	58	55	12	1	0	0
	25	45	57	65	71	51	14	1	6	0
	50	42	27	12	73	19	11	1	3	0
Room temp. (dark)	$CaCl_2$	82	85	82	94	65	2	3	0	0
	0	14	0	0	0	0	0	0	0	0
	5	79	51	0	40	33	1	3	0	0
	10	90	99	5	93	86	3	2	4	0
	15	92	99	56	90	81	7	8	5	0
	25	93	95	36	92	76	6	2	3	0
	35	89	93	38	94	81	6	6	4	0
	50	25	32	31	88	45	3	4	1	0
	75	0	0	0	0	0	0	0	0	0
2° C. (dark)	0	31	76	0	73	1	19	1	0	0
	5	93	99	5	96	62	46	52	14	31
	10	98	98	19	97	96	63	60	14	34
	15	81	97	71	99	87	54	53	21	41
	25	89	99	95	98	86	43	59	32	46
	35	94	99	69	98	95	43	47	38	46
	50	88	98	94	98	93	46	50	50	11
	75	93	92	91	94	99	42	57	58	0
Av. germination		66.5	72.7	37.5	77.3	60.6	21.0	2.5	12.7	10.4

All pine and spruce species tend toward uniformity in optimum humidity requirements at room temperature (dark), namely 15 to 35%. However, at 2° C., optimum humidity is roughly in the range 25 to 75% for pine, with the spruce forms having individual optimum requirements, as follows: Norway spruce, 10%; white spruce No. 1, 5% to 75%; and white spruce No. 2, 75%.

These results agree very well with those of Duffield and Snow (2) who found that pollens of white and red pines retained vitality best at 50% relative humidity and 0° to 40° C., and with those of Nebel and Ruttle (4) who found that pollens of fruit trees kept best at 50% relative humidity and 2° to 8° C. From Table VII it would appear that, for white pine and white spruce No. 2, 75% relative humidity was superior to 50%. That relative humidities of the order of 50 to 75% should provide optimum storage conditions for pollen is perhaps surprising, since for many years it had been popularly supposed that pollen should be stored under relatively dry conditions.

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STUDIES ON THE MICROBIOLOGY OF RECONTAMINATED STERILIZED SOIL IN RELATION TO ITS INFESTATION WITH *OPHIOBOLUS GRAMINIS* SACC.[1]

By R. A. Ludwig[2] and A. W. Henry[2]

Abstract

In these studies recontaminated steam-sterilized black soil, when infested with the take-all fungus, *Ophiobolus graminis*, gave, as a rule, less severe infection of wheat seedlings than similarly infested unsterilized soil. One explanation suggested is that the microflora that develops in sterilized soil following recontamination has a greater suppressive action on *Ophiobolus graminis* than that normally present in unsterilized soil. These two microfloras were found to differ both quantitatively and qualitatively. In general the number of microorganisms, as determined by the plate count method, was found to be much greater in sterilized recontaminated soil than in unsterilized soil. The fact that the fungus *Trichoderma viride* develops rapidly and becomes a dominant organism in sterilized recontaminated soil is considered to be of especial significance. The antagonism of this fungus toward *Ophiobolus graminis* probably plays an important role in suppressing the latter.

Introduction

A great deal of the experimental work with *Ophiobolus graminis* has been conducted in pots of sterilized soil artificially infested with the fungus. It has already been demonstrated (5) that such experiments do not necessarily give a reliable measure of the behaviour of this fungus in normal unsterilized soil. Further evidence of this fact was given by preliminary observations that indicated that wheat seedlings were less severely attacked by *Ophiobolus graminis* in sterilized soil that was recontaminated with a little unsterilized soil than they were in unsterilized soil. The experiments reported in this paper verify these preliminary observations and demonstrate some of the changes that may be responsible for this reduced severity of attack.

The Comparative Reaction of Wheat Seedlings to *Ophiobolus graminis* in Sterilized Recontaminated Soil and in Unsterilized Soil

Previous Work

Differences in the reaction of wheat seedlings to *Ophiobolus graminis* in sterilized and unsterilized soil have been reported by Henry (5). He showed that at medium and high temperatures infection of wheat was more severe in sterilized than in unsterilized soil. In his work, however, inoculum was added and the seed was sown immediately after sterilization of the soil.

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Methods and Results

A summary of the results of two experiments to compare the behaviour of *Ophiobolus graminis* in sterilized recontaminated soil and in unsterilized soil is reported below.

The inoculum of *Ophiobolus graminis* used in these experiments was grown in 200-ml. Erlenmeyer flasks containing a mixture of 45 gm. of air-dry black loam and 5 gm. of corn meal, moistened with 27 ml. of tap water. These flasks were sterilized by autoclaving for two hours at 15 lb. pressure, sown with *Ophiobolus graminis*, and incubated for three to four weeks before using.

Both experiments were conducted in 6-in. pots, a soil mixture consisting of three parts black loam and one part sand being used. The soil for all pots was prepared at one time to insure uniformity. The soil was sterilized by autoclaving in the pots for eight hours at 15 lb. pressure. On cooling it was immediately recontaminated by sprinkling the surface of the sterile soil with a few grams of unsterilized soil and working the latter in to a depth of about an inch with a sterile spatula.

In planting, the surface inch of soil was removed with a sterilized scoop, the required amount of inoculum was added and 25 seeds of good quality Red Bobs wheat sown on it. The seed was then covered by returning the surface soil to the pot. All pots included in the experiment were then randomized in a block on a single greenhouse bench. During the experiment the temperature was maintained as close to 20° C. as possible.

TABLE I

THE COMPARATIVE REACTION OF WHEAT SEEDLINGS TO *Ophiobolus graminis* IN STERILIZED RECONTAMINATED AND IN UNSTERILIZED SOIL

Experiment	Average plant height, cm.	Dry weight per pot, gm.	Degree of infection, %
I. Average of all treatments (Rates of inoculation and periods of recontamination prior to sowing)			
Sterilized recontaminated (48)*	17.2	—	59.3
Unsterilized (48)	9.8	—	77.5
Difference required for significance	6.0	—	—
II. Average of all treatments (Various organic amendments applied singly and in combination with ammonium phosphate to unsterilized and sterilized recontaminated soil)			
Sterilized recontaminated (64)	29.8	0.96	36.5
Unsterilized (64)	24.5	0.75	52.7
Average of control (The non-fertilized series from above)			
Sterilized recontaminated (8)	24.9	0.85	52.9
Unsterilized (8)	16.7	0.60	81.0
Difference required for significance (8)	3.29	0.121	11.85

* *Figures in parentheses indicate the number of pots included in the averages.*

The plants were allowed to grow for three to four weeks before notes were taken. In taking notes the height of each plant was measured and a numerical rating of its disease reaction was made. Following this, in one experiment, the roots were removed and the dry weight of the tops was determined for each pot.

The data from the two experiments are summarized in Table I. In Experiment I each figure represents an average of the data from 48 pots. These 48 pots were divided into six treatments of eight pots each. The treatments consisted of two rates of inoculation, namely, 25 and 50 gm. per pot, and three periods of varying length between recontamination and inoculation. All pots were sown on the same date. In Experiment II each figure under "average of all treatments" represents an average of the data from 64 pots, while each figure under "average of control" represents an average of the data from eight pots. Experiment II was a fertilizer experiment* in which dried green alfalfa, mature wheat straw, and poplar sawdust were mixed with the soil singly and in combination with ammonium phosphate. These materials were applied to unsterilized and sterilized recontaminated soil. Photographs of representative pots from the control series, which received no fertilizer treatment, are shown in Fig. 1.

Each of the three methods of recording disease severity points to the same conclusion, namely, that the wheat seedlings are less severely attacked by *Ophiobolus graminis* in sterilized recontaminated soil than they are in unsterilized soil. The degree of infection in Experiment I was decreased by 18.2%, while in the control series of Experiment II it was decreased by 28.1%.

Discussion

From the results given in the preceding table and from other similar data not reported here, it is concluded that, in general, less severe infection of wheat seedlings by *Ophiobolus graminis* occurs in black loam that has been sterilized and then recontaminated with a little unsterilized soil, than in the unsterilized soil itself. Henry (3, 5) has shown that the microflora of unsterilized soil exerts a certain suppressive action on fungi that cause basal rots of cereals, an effect that obviously could not occur in uncontaminated sterilized soil. There is, then, progressively less infection of wheat seedlings by *Ophiobolus graminis* in sterilized soil, unsterilized soil, and sterilized recontaminated soil.

A number of hypotheses might be advanced to explain such effects. A plausible one is based on the known antagonistic effects (3, 10) of various members of the natural microflora of the soil. These might suppress a pathogen in several ways, e.g. by exhausting available food supplies, by excreting toxic or lytic substances, or by actually parasitizing it. In sterilized recontaminated soil any or all of these effects may be increased by a change in the number and activity of the micro-organisms or by a change in the

* *We are indebted to the Department of Soils, University of Alberta, for co-operation in the conduct of this experiment.*

dominant species present. Studies were therefore undertaken to compare the microfloras of sterilized recontaminated and unsterilized soil from both a quantitative and a qualitative point of view.

Comparative Studies on the Microbial Populations of Sterilized Recontaminated and Unsterilized Soil

Quantitative Changes

Previous Work

The fact that tremendous increases in the number of micro-organisms follows partial sterilization of the soil (9, 11, 12) has been known for a long time. Russell and Hutchinson (9) found from five to nine million bacteria in untreated soil and forty million in soil that had been partially sterilized. Partial sterilization differs from complete sterilization and recontamination in that, in the recontaminated soil all types of micro-organisms originally present are returned to the soil after treatment, whereas in the former only those resistant to the partial sterilization treatment remain.

Methods

The numbers of bacteria, fungi, and actinomycetes were determined by the plate count method. Black loam was collected at a depth of 2 to 3 in. after the careful removal of the surface soil. One thousand grams of the moist soil so collected were placed in each of four 2000 ml. Erlenmeyer flasks, the moisture content was determined and made up to 23%. This moisture level was maintained throughout the experiment by additions of sterile water. Two of the flasks were autoclaved for four hours at 15 lb. pressure, and after cooling were recontaminated with a small amount of the original soil. A sampler, by means of which a core of soil $\frac{5}{8}$ in. in diameter and 2 to $2\frac{1}{2}$ in. long could be removed without contamination, was devised. The extreme edges of the soil mass were avoided in drawing samples. Plating was done at intervals, one sample from each flask being plated each time. Immediately on removal from the flask the sample was dropped into a sterilized, cooled, and weighed water blank in a shaker bottle. The weight of the sample was then determined by reweighing the bottle. Since relative and not absolute figures were desired, the numbers were computed on the basis of a gram of moist soil.

The method of plating used was kindly recommended by James (6). Czapek's medium (2, p. 13) with 17.5 instead of 15 gm. of agar per litre was employed for fungi. This medium was poured into flasks, 100 ml. per flask, and acidified at the time of using with $\frac{1}{2}$ ml. of a solution of 10 ml. of 85% lactic acid in 100 ml. of water. For bacteria and actinomycetes, sodium albuminate agar (2, p. 9) was employed. A 100 ml. flask of each medium was used to pour 10 plates. The numbers of bacteria and actinomycetes were determined in the same plates. All plates were incubated at 25° C. The fungal counts were made after four days, the bacterial after eight days, and the actinomycete after 12 days. Where there was any doubt as to the nature of the colony, it was examined with a microscope.

TABLE II

COMPARATIVE NUMBERS OF FUNGI, BACTERIA, AND ACTINOMYCETES IN STERILIZED RECONTAMINATED AND UNSTERILIZED EDMONTON BLACK LOAM, PER GRAM OF MOIST SOIL

Number of days	Fungi			Bacteria			Actinomycetes		
	Sterilized recontaminated	Unsterilized	Difference	Sterilized recontaminated	Unsterilized	Difference	Sterilized recontaminated	Unsterilized	Difference
0	4	35.1×10^3	-35.1×10^3	1439	18.6×10^6	-18.6×10^6	115	1.1×10^6	-1.0×10^6
2	50	23.4×10^3	-23.4×10^3	4.6×10^6	7.2×10^6	-2.6×10^6	0	2.0×10^6	-2.0×10^6
4	76.8×10^3	26.2×10^3	50.5×10^3	166.1×10^6	12.7×10^6	153.4×10^6	0	2.1×10^6	-2.1×10^6
8	90.0×10^3	20.0×10^3	70.0×10^3	188.0×10^6	5.4×10^6	182.6×10^6	1×10^6	2.5×10^6	-1.5×10^6
13	550.5×10^3	26.8×10^3	523.7×10^3	177.9×10^6	6.5×10^6	171.4×10^6	1.1×10^6	3.7×10^6	-2.6×10^6
17	77.0×10^3	18.6×10^3	58.4×10^3	118.8×10^6	6.4×10^6	112.4×10^6	3.3×10^6	3.7×10^6	-0.4×10^6
25	93.0×10^3	27.6×10^3	65.4×10^3	160.3×10^6	11.8×10^6	148.5×10^6	4.6×10^6	4.2×10^6	0.4×10^6
57	224.5×10^3	30.2×10^3	194.3×10^3	135.6×10^6	9.5×10^6	126.0×10^6	14.1×10^6	5.1×10^6	9.0×10^6
80	86.8×10^3	39.3×10^3	47.6×10^3	61.4×10^6	4.5×10^6	56.9×10^6	10.4×10^6	3.0×10^6	7.4×10^6

Results

Data showing the comparative numbers of fungi, bacteria, and actinomycetes, computed on the basis of a gram of moist soil, are given in Table II. Counts of each of the three groups remained relatively constant in unsterilized soil, both between different dates of plating and between duplicate flasks, while in the sterilized recontaminated soil considerable fluctuation occurred between different dates and also between flasks. All counts show, however, that there is a tremendous increase in both the numbers of bacteria and fungi after an initial incubation period. An increase also occurred in the numbers of actinomycetes but this was less marked than that of the fungi and bacteria.

Discussion

The plate count method is open to criticism on the grounds that only members of the particular group of soil organisms that will grow on the medium chosen are obtained. Further, in the case of fungi, colonies obtained are largely of single spore origin and consequently profusely sporing types will appear more often in the plates than those that sporulate poorly or not at all.

In spite of these objections, it is felt that results indicate increased numbers in at least certain groups of fungi. While the relative numbers of different fungi are largely proportional to the spore producing ability of the various types, there is also a great increase in vegetative growth. Profuse mycelial growth can often be observed in sterilized recontaminated soil. The comparatively small number of fungi obtained from sterilized recontaminated soil after an incubation period of two days (Table II) may be attributed to the fact that the fungi have not had sufficient time to produce spores.

Large increases in bacterial numbers have also been demonstrated. The absence of actinomycetes in early platings may be attributed to the fact that extremely high dilutions had to be used for bacterial counts and the slower

growing organisms were lost in the dilution. Though it appears that this group is not as important as the bacteria and fungi, the fact that certain individuals may exert striking antagonistic effects should not be ignored.

The large increase in numbers of micro-organisms may well be a factor contributing to the more marked inhibition of *Ophiobolus graminis*. This increase in numbers would result in increased competition for the pathogen. Either the new soil population as a whole or certain individuals predominating in the new population may be responsible for this effect.

Qualitative Changes

Previous Work

In addition to an increase in the numbers of micro-organisms following steam sterilization and recontamination, the kinds of micro-organisms predominating may differ from those occurring in unsterilized soil. This has been shown to be the case in partially sterilized soil. Russell and Hutchinson (9), for example, observed that in heated soils the new flora was different from that in untreated soil. In toluened soil they found the new flora to be the same as the old. Waksman and Starkey (11, 12) recorded that bacterial numbers increased markedly in soil treated with a small amount of toluene, while the number of fungi decreased. The treatment is reported to have made the soil a much better medium for growth so that those fungi that survived, namely, *Zygorhynchus*, *Penicillium*, *Cunninghamella*, and *Cephalosporium* increased in numbers.

As has already been pointed out, however, partially sterilized soil differs from sterilized recontaminated soil in that, in the latter, all the organisms originally present are returned after treatment, while in the former only those resistant to the sterilization treatment remain.

Methods

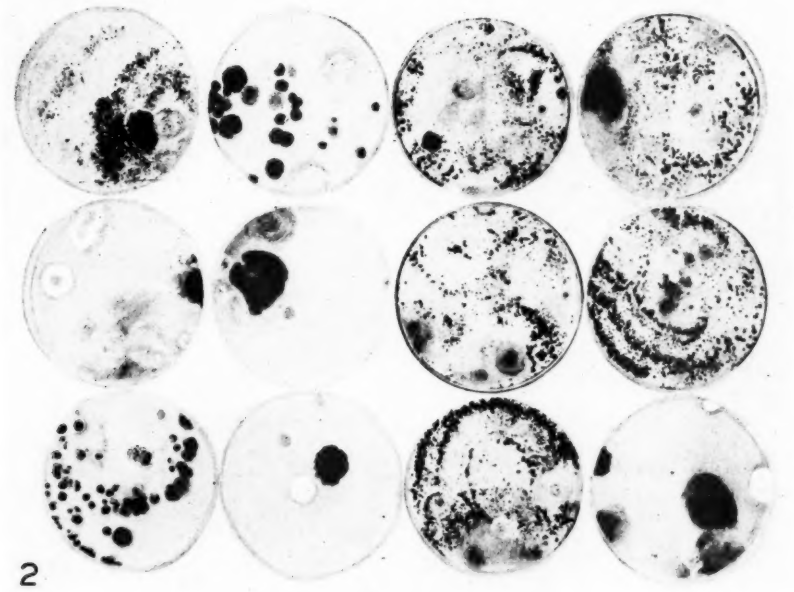
The results reported here were obtained from the plates used in the quantitative work just reported and from other similar experiments. The results of platings from pot as well as flask culture are included. At the time of making counts most of the colonies were small. These were allowed to develop further and the fungi to sporulate before notes as to the kind of organism were made.

Results

In platings of unsterilized soil the following genera of fungi, arranged in order of decreasing prominence were obtained with greatest frequency:—*Penicillium* (a number of distinct species), *Macrosporium*, *Aspergillus*, *Rhizopus*, and *Zygorhynchus*. A variety of other fungi such as *Trichoderma* and *Fusarium* as well as numerous unidentified types were occasionally obtained. The *Penicillium* species were by far the most prominent.

Early platings of sterilized recontaminated soil yielded mostly *Penicillium* and *Rhizopus*. These appeared to be the same as those found in unsterilized soil. Following an incubation period of 8 to 10 days, however, *Trichoderma*

PLATE I



EXPLANATION OF FIGURES

FIG. 1. *The comparative reaction of wheat seedlings to Ophiobolus graminis in sterilized recontaminated and unsterilized soil. Left to right:—unsterilized soil, unsterilized soil plus Ophiobolus, sterilized recontaminated soil plus Ophiobolus, and sterilized recontaminated soil.*

FIG. 2. *Relative prevalence of Trichoderma in sterilized recontaminated and unsterilized soil. Right:—platings from six pots of sterilized recontaminated soil showing the occurrence of Trichoderma in all except one, that represented by the plate in the lower right hand corner. Left:—platings from six pots of unsterilized soil showing the occurrence of Trichoderma in only one, that represented by the plate in the upper left hand corner.*

became the predominating genus and remained so after 80 days, the end of the most extended series of platings. Once this fungus appeared it became difficult to study other genera, since the plates were rapidly overgrown by it. *Trichoderma* sp. was almost invariably obtained from platings of incubated pots and flasks, even though it only occasionally appeared in platings of unsterilized soil. Fig. 2 shows a series of plates from the soil of the pots illustrated in Fig. 1 (also average of control, Table I). This figure shows the presence of *Trichoderma* sp. in five of the six pots of sterilized recontaminated soil that were tested and in only one of the six pots of unsterilized soil that were tested. A culture of the organism was sent to Dr. Thom of the United States Department of Agriculture, Washington, D.C., who kindly confirmed the identification as to genus. Although no reference was made by Dr. Thom to species, it is quite evidently *T. viride*, as described by Bisby (1).

No striking differences in the types of bacterial colonies from unsterilized and sterilized recontaminated soil were observed. However, since no detailed study was made of them it is impossible to conclude anything concerning the presence or absence of qualitative change. The same may be said for the actinomycete colonies obtained.

Discussion

The most significant qualitative difference noted between the microfloras of recontaminated soil and unsterilized soil was the greater prevalence of *Trichoderma viride* in the former. Its presence is thought to be an important contributing factor to the reduced severity of attack by *Ophiobolus graminis* in sterilized recontaminated soil (Fig. 1). The antibiotic action of various species of *Trichoderma*, particularly *T. viride (lignorum)*, on a number of important plant pathogens has been extensively studied. A summary of this work as well as an excellent bibliography may be found in Porter and Carter's review (8).

The failure to isolate *Trichoderma* regularly from unsterilized soil may be due to its inability to fruit abundantly when in competition with numerous other micro-organisms as has been shown for *Helminthosporium sativum* (4). It is widely prevalent in natural Alberta soils as will be demonstrated in a later paper, but does not predominate there to the extent that it does in recontaminated sterilized soil. In Manitoba it was found by Bisby *et al.*, as reported by Bisby (1), to constitute 7% of the fungi that they isolated from natural soil.

General Discussion

A comparative study of the microfloras of sterilized recontaminated and unsterilized soil has revealed marked differences in numbers and kinds of micro-organisms in the two. Such differences may well explain why *Ophiobolus graminis* is suppressed more in the former soil medium than in the latter, as is demonstrated. Just how this is accomplished is not definitely known. The increased numbers or activity (7), may play a part by exhausting essential food material needed by the pathogen, by producing toxic substances or in

other ways. The qualitative changes may be even more important, giving known antagonists such as *Trichoderma viride* special encouragement. A number of effects such as these may be and probably are operating simultaneously, so that it may well be their combined effect that is manifesting itself. If, under field conditions, the microbiological population of the soil could be adjusted to approximate that of sterilized recontaminated soil, it should be possible to reduce damage caused by *Ophiobolus graminis* to wheat and other susceptible plants.

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DRIED WHOLE EGG POWDER

VIII. AN IMPROVED FLUORESCENCE METHOD AND SOME FACTORS AFFECTING THE MEASUREMENT[1]

BY J. A. PEARCE[2], M. W. THISTLE[2], AND MARGARET REID[2]

Abstract

An improved method avoided prolonged filtration periods and effected a material saving in chemicals, but the results do not differ from those obtained by the original method. The improved method is as follows: 2.5 gm. of egg powder is defatted with three 25 ml. portions of chloroform; 1 gm. of the defatted powder is shaken for 30 min. with 100 ml. of 10% sodium chloride, filtered, and the fluorescence of 15 ml. of the filtrate determined in a photofluorometer.

Increase in temperature raised the values obtained by 0.65 photofluorometer units per Centigrade degree. Although decrease in the pH of the protein extracts was associated with increase in fluorescence values, variations in the pH of the protein solvent from 4.6 to 8.9 caused no significant change in fluorescence values.

Introduction

A fluorescence method as applied to dried whole egg powders was observed to be related to flavour quality (2, 3) and has been applied satisfactorily in the investigation of factors affecting quality (4, 5). However, the method was somewhat laborious for routine control work, mainly because of an excessively long filtering period. This paper describes an improved method designed to overcome these difficulties; and to give results comparable to those obtained by the original method. In addition, observations were made on the effect of temperature of extraction and pH of the protein solvent on the fluorescence values obtained.

Initial Experiments

Extraction of Fat

Some 13 fat solvents were tested in earlier work (1, 2). Of these, ethyl ether (1) appeared to warrant further study, as it gave equivalent readings and was considered somewhat less toxic than chloroform. Six samples, graded from 4 to 9 on a palatability scale (2, 3) were tested by the original method (2), except that the fat solvents and the number of fat extractions were varied.

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[2] *Biochemist, Food Investigations.*

Table I shows that, on the average, fluorescence values after two extractions did not differ significantly from those after three extractions when chloroform was used, and that the values obtained by three extractions with ethyl ether

TABLE I

EFFECT OF FAT SOLVENT AND NUMBER OF EXTRACTIONS ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Fat solvent	Mean fluorescence value
Chloroform	
Two extractions	39.85
Three extractions	38.65
Ethyl ether	
Two extractions	41.59
Three extractions	39.28
Necessary difference, 5% level of statistical significance = 1.48	

did not differ significantly from the values obtained with chloroform. However, ethyl ether was found to be much less convenient to use experimentally, and was given no further consideration.

Extraction of Protein

The original method (2) was based on an assumed total extraction of the fluorescent materials from the defatted powder. The first attempt at an improved procedure tested the equilibrium principle of extraction on six samples, as follows: 2.5 gm. of defatted powder was shaken with 100 ml. of 10% potassium chloride solution for periods of 5, 10, 15, 30, 60, and 120 min., made up to 250 ml. with 10% potassium chloride solution, mixed, filtered, and the fluorescence of the filtrate determined in the Coleman photofluorometer. Sufficient filtrate for reading purposes was obtained in 10 to 15 min. Table II shows that this method gave results significantly higher than those

TABLE II

EFFECT OF EXTRACTION TIME ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Method	Mean fluorescence values	
	Modification No. 1[1]	Modification No. 2[1]
Original (complete extraction)	41.62	40.82
Modified (equilibrium extraction)		
5 min.	43.52	—
10 min.	45.10	—
15 min.	44.63	—
30 min.	46.35	38.78
45 min.	—	40.93
60 min.	47.54	45.56
120 min.	46.66	—
Necessary difference, 5% level of statistical significance	1.75	2.29

[1] *Modification described in text.*

obtained by the original method. However, the data were of value in indicating maximum extraction of fluorescent materials in about 30 to 60 min. The greatly reduced filtering period suggested that a somewhat similar procedure might be satisfactory.

In a second attempt to utilize the equilibrium method of extraction, six samples were treated as follows: 1 gm. of defatted powder was transferred to a 250 ml. Florence flask, 100 ml. of 10% potassium chloride solution added, the mixture shaken for periods of 30, 45, and 60 min., filtered, and the fluorescence of the filtrate determined. Table II indicates that when the samples had been shaken for periods of 30 and 45 min., the results did not differ significantly from those obtained by the original method. The results from powders shaken for 60 min., in addition to differing significantly from the control method, did not agree closely between duplicate measurements. It would therefore appear that the equilibrium method of extraction for 30 to 45 min. can give satisfactory results.

Although 10% potassium chloride is commonly used as a protein solvent, previous work (1) had indicated 10% sodium chloride as a possible substitute. Since sodium chloride is cheaper and more easily obtainable than potassium chloride, both solvents were tested on six samples of powder, using the second modification (above) with 45 min. shaking. Table III shows no significant

TABLE III

EFFECT OF PROTEIN SOLVENTS (10% SOLUTIONS) AND METHOD OF EXTRACTION ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

Method	Mean fluorescence value
Original	
Potassium chloride	40.82
Sodium chloride	44.14
Modification No. 2[1]	
Potassium chloride	40.94
Sodium chloride	41.25

Necessary difference, 5% level of statistical significance = 1.73

[1] *Modification described in text.*

difference between the fluorescence values when sodium and potassium chloride were used in the modified procedure. No difference was evident between the new method, using either solvent, and the original method using potassium chloride, the results for the second modification in Table II thus being supported.

The effect of rate of shaking was evaluated on four egg powders of varying quality as follows: the defatted powders were extracted with sodium chloride solution by shaking at 100, 190, 290, and 390 impulses per minute (complete cycles) for 10, 20, 30, 40, 50, and 60 min. The effect of shaking time was

similar to that recorded in Table II. Mean fluorescence values were 40.4, 41.2, 42.7, and 43.5 for the shaking speeds noted. Although fluorescence appears to increase with increasing rates of shaking, these differences were not significant in comparison with the replicate error. Accordingly, 290 impulses per minute was chosen as a convenient rate.

Main Experiment

The foregoing preliminary experiments indicated that the number of fat extractions, the substitution of sodium for potassium chloride as protein solvent, and the length of the shaking period all merited further investigation. Consequently a more rigorous test was conducted on 16 powders consisting of two samples, each representing a carload lot of powder, prepared for export by each of eight Canadian egg-drying plants. All possible combinations of these factors were tested as follows: two and three defattings with chloroform, 10% potassium and sodium chloride solutions, the effect of 30 and 45 min. shaking by the equilibrium extraction method, and comparison of the equilibrium method with the original method.

Upon inspection of the results (Table IV) it was evident that the product from the four plants with the highest production rates was more variable than that from the remaining four plants. Therefore, in order to assess the significance of the various treatment differences, the plants were divided into two groups for purposes of statistical analysis. The only modified procedure

TABLE IV

EFFECT OF VARYING THE NUMBER OF DEFATTINGS, THE PROTEIN SOLVENT, AND THE METHOD OF PROTEIN EXTRACTION ON THE FLUORESCENCE VALUES OF 16 EGG POWDERS FROM EIGHT CANADIAN DRIED EGG PRODUCERS

Protein solvent	Procedure	Number of defattings with chloroform	Mean fluorescence values for groups of four plants	
			Group I	Group II
10% sodium chloride	Original	2	19.61	18.79
		3	17.20	16.80
	Modified, 45 min. shaking	2	19.41	19.06
		3	18.02	17.40
	Modified, 30 min. shaking	2	24.48	23.24
		3	16.94	17.26
10% potassium chloride	Original	2	17.59	17.28
		3	16.72	16.34
	Modified, 45 min. shaking	2	18.85	19.16
		3	18.16	18.04
	Modified, 30 min. shaking	2	20.79	20.01
		3	18.62	17.71
Necessary difference, 5% level of statistical significance			1.07	1.43

common to both groups that did not differ significantly from the original method involved three defattings with chloroform and protein dispersion by 30 min. shaking in 10% sodium chloride solution.

Modified Procedure

As a result of the foregoing work, an improved procedure is suggested for measuring the fluorescence of dried whole egg powder. According to this procedure, 2.5 gm. of egg powder is defatted at room temperature with three 25 ml. portions of chloroform (corks or stoppers are unsuitable), and filtered each time through a No. 1 Whatman filter paper. A fresh filter paper is used for the third extraction. After the final extraction, the defatted powder is allowed to dry at room temperature (about an hour), 1 gm. of defatted powder is transferred to a 250 ml. flask, and 100 ml. of 10% sodium chloride added. The mixture is shaken for 30 min. at approximately 300 impulses per minute, and filtered through No. 1 Whatman filter paper. The fluorescence of a 15 ml. portion of the filtrate, usually obtained in 10 to 15 min., is determined in the Coleman photofluorometer.

The Effect of Temperature and pH on Fluorescence Values

Temperature

During earlier work (1, 2, 3) it was observed that temperature of extraction had an effect on the fluorescence value. The effect of temperature on both the fat and protein extractions was assessed by carrying out these operations at temperatures varying from 3.6° to 22.8° C., the remainder of the procedure being done at room temperature (23 to 25° C.). The results are given in Table V. The temperature coefficient for the defatting operation was 0.30

TABLE V

EFFECT OF TEMPERATURE OF FAT AND PROTEIN EXTRACTIONS ON FLUORESCENCE VALUES

Temperature, °C.	Fluorescence values	
	$CHCl_3$ extraction	KCl extraction
22.8	46.8	43.5
15.6	44.5	40.0
5.0	41.0	36.9
3.6	40.2	35.2

photofluorometer units per Centigrade degree, while that of the protein extraction was 0.35 units. Thus it may be seen that increase in temperature of 1° C. caused an over-all increase in the fluorescence value of 0.65 units. (1° F. change alters the value by 0.36 units.)

Day to day variations in laboratory temperature could, therefore, be expected to have some effect on the results. It is recommended that laboratory temperature be kept as nearly constant as possible, or failing this, that suitable corrections be made for temperature variations.

pH

It was previously noted that a relation existed between the pH of potassium chloride and water extracts of egg powder and the fluorescence value (3). To investigate this, the pH of aqueous extracts of fresh and stored egg powder was varied by means of hydrochloric acid and sodium hydroxide, and that of 10% potassium chloride extracts of similar stored powder with acetic acid and ammonium hydroxide. After the fluorescence at the altered pH values had been determined, an equivalent amount of acid or base was added to restore the pH to approximately its initial value, and the fluorescence again measured.

The results (Table VI) show that the fluorescence values varied with pH. Maximum values occurred in extracts of fresh powder at pH 2.5; in the stored powders, at pH 4.8 and 4.2. Between pH 1.0 and 10.0 the changes

TABLE VI

EFFECT OF CHANGING THE pH OF PROTEIN EXTRACTS ON FLUORESCENCE VALUES

Water extracts				10% KCl extract	
Sample No. 1		Sample No. 2		Sample No. 3	
pH	Fluorescence value[1]	pH	Fluorescence value[1]	pH	Fluorescence value[1]
(1 *N* acid)	27.5	(1 *N* acid)	27.5	(1 *N* acid)	67.6
1.21	11.2	1.20	32.9	3.26	35.0
2.48	25.2	2.51	47.0	3.83	49.4
3.36	17.7	3.88	51.3	4.18	57.9
4.29	11.5	4.82	73.8	4.39	47.0
5.42	11.2	6.19	47.5	5.05	37.0
6.92[2]	9.9[2]	8.15[2]	35.0[2]	6.29[2]	34.8[2]
7.23	10.3	8.18	35.4	9.88	34.8
9.36	11.0	9.78	27.7	10.12	34.8
				10.41	34.8
				10.60	32.8
				10.96	31.8
(1 *N* base)	7.0	(1 *N* base)	11.5	(1 *N* base)	67.4

[1] *Blank corrections for solvent applied (1).*
[2] *Extract without added acid or base.*

in fluorescence values were substantially reversible, e.g. the addition of an equivalent quantity of base to Extract 1 (Table VI) at pH 3.36 resulted in a fluorescence value of 10.0; the same extract containing the same amount of sodium chloride also had a fluorescence value of 10.0. However, reversibility was not obtained when extracts were made as strongly acidic or basic as 1 *N*.

From the foregoing, it might be assumed that variations in the pH of the water used in preparing the protein solvent might affect the fluorescence value. However, variations in the pH of the protein solvent from 4.6 to 8.9 had no significant effect on the fluorescence values of egg powders of widely varying quality (Table VII).

TABLE VII

EFFECT OF VARIATION IN pH OF SODIUM CHLORIDE SOLUTIONS ON FLUORESCENCE VALUES OF SIX SELECTED EGG POWDERS

pH of solvent	Mean fluorescence value
4.6	37.53
5.2	38.92
5.6[1]	38.65
8.9	39.45

[1] *pH of the distilled water used.*

Thus, altering the pH of the protein solvent has very little effect, whereas changing that of the extracts themselves does affect the values obtained. This behaviour might be explained on the basis of a "pseudo-buffering" effect of egg powder in protein solvents. It was found that egg powder with a pH of 7.13 in sodium chloride solution, when dispersed in sodium chloride plus sodium hydroxide solution with pH 8.50, assumed a resultant pH of 7.16. Therefore it seems unlikely that the range of pH ordinarily observed in laboratory distilled water will have any appreciable effect on the magnitude of the fluorescence value.

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INVESTIGATIONS ON THE USE OF IRISH MOSS IN CANNING OF MEAT[1]

By E. J. Reedman[2] and Leonard Buckby[3]

Abstract

In an attempt to develop a suitable substitute for the agar used in large quantities by the canning industry, gelose preparations were made from bleached and unbleached Irish moss. About 30 to 60% of the total moss solids, depending on the type of moss used, were extracted in one-half hour by hot water. Suspended solids were removed by filtration with diatomaceous earth, and the extracts purified with activated charcoal. Extracts were dried successfully on a laboratory model double-drum drier, an experimental spray drier, an experimental tunnel drier, and by air-drying the concentrated extract at room temperature. All methods of drying yielded products capable of forming good jellies.

Although agar jellies were definitely stronger than those from equivalent concentrations of Irish moss, the addition of 0.2% potassium chloride produced jellies from Irish moss that were stronger than those from agar. On the basis of consumer taste panel tests on canned chicken, no statistically significant difference could be demonstrated between the acceptability of agar and Irish moss jellies.

Introduction

The scarcity of agar due to wartime conditions has created a need for a substitute material. This is particularly true in the canning industry, which was one of the largest consumers of agar. In these circumstances algae other than the agar-producing species appeared to be worthy of investigation as possible sources of substitute materials.

Irish moss *(Chondrus crispus)* was chosen for study since it grows in large quantities in the waters of the Canadian east coast. It has already proved to be a valuable suspending and clarifying agent in several industries, either as an extract or the ground entire plant. The plant grows in shallow waters and is harvested from May to October by a raking process. Moss spread on the beach, allowed to dry and baled immediately is known as unbleached or black moss; bleached moss is allowed to lie exposed to the sun until a fairly uniform cream colour is produced. During the bleaching the moss is washed several times with sea water. Samples of such moss, from the east and west coasts of Prince Edward Island, were used in the present investigation, with the purpose of developing a method for the preparation of gelose from Irish moss and investigating the properties requisite for its use in canning of meat.

Procedure

Preparation of Sample

The type and origin of the moss samples used are indicated in Table I. Fresh moss was shipped wet to the laboratory, ground moss was produced by grinding whole air-dry plants in a Wiley mill through a coarse screen, and

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TABLE I

YIELD AND PROCESSING DATA FOR IRISH MOSS EXTRACTIONS

Type, treatment, and origin of moss	No. of preparations examined	First extract				Second extract
		Conc., % solids	Soluble solids, % of total moss solids	pH	Viscosity at 100° F., time in sec. (Water = 34 sec.)	Soluble solids, % of total moss solids
Fresh moss						
Whole	1	0.41	50	7.73	36.8	10
Dried unbleached moss						
Whole (West Shore, Bag 1)	2	0.37	47	7.54	41.4	9
Ground (East Shore, Bag 3)	2	0.42	54	7.55	42.9	8
Leached (West Shore, Bag 1)	3	0.23	29	7.31	41.6	17
Dried bleached moss						
Whole (East Shore, Bag 2)	4	0.47	59	7.27	54.9	9
Ground (East Shore, Bag 2)	2	0.42	50	7.45	56.0	10
Leached (East Shore, Bag 2)	2	0.33	40	7.24	45.9	17

leached moss was prepared by washing in cold running water (below 5° C.) for three days. It was found that about 18% of the weight of unbleached moss was lost by leaching but only 3% of bleached moss was leached out. A considerable part of the weight lost by unbleached moss consisted of extraneous material such as sand. The bleached moss was invariably much cleaner.

Extraction

Since the gelose is readily soluble in hot water its extraction is not difficult. However, several methods were investigated. Only small amounts of gelling substances were extracted by cold dilute sodium hydroxide or cold dilute hydrochloric acid, and hydrolysis occurred in both instances when heat was applied.

Preliminary studies indicated that one-half hour extraction with hot water was the best practical procedure, further extraction being effected by increasing the proportion of water rather than by lengthening the time of extraction. However, further dilution adds to the difficulty of drying the extract. The extracts were prepared as follows: 150 gm. of the dry plant (containing 6 to 10% moisture) was extracted for one-half hour in 20 litres of water with constant stirring in a steam jacketed kettle. The temperature of the steam in the jacket was maintained at 100° C. The extracts were strained through a 30-mesh screen with stirring but without pressure. Further information was obtained by extracting the screened residue with another 20 litres of water for one hour and straining as before. This second extract was weighed, and after samples were taken for solids determinations, it was discarded.

The extract from leached moss was identical with the "hot extract" described by Haas and Hill (2).

Results listed in Table I show that the first extract contained soluble solids representing from 29 to 59% of the dry weight of original moss. Soluble solids in the second extract varied from 8 to 17% of the weight of the original moss. Data given are means of several extractions.

Filtration

The screened extract had undesirable colour, odour, and taste, and contained some suspended solid. Purification was accomplished by treatment with activated charcoal and filtration through diatomaceous earth. Since many of the extracts were quite viscous, difficulty in filtration set an upper limit on the amount of solids an extract might contain. Although unbleached moss gave filterable extracts at higher original concentrations than 150 gm./20 litres water, this was the maximum concentration that could be handled conveniently for bleached moss, and therefore was adopted as standard procedure throughout.

Purification entailed two steps: first, the removal of suspended solids; and second, the removal of colour, odour, and taste with activated charcoal. Attempts to remove all impurities by one filtration step proved impracticable with small-scale equipment. The filter press used was a four plate aluminum laboratory size model of standard design with eight filtering surfaces $4\frac{7}{8} \times 4\frac{7}{8}$ in. Canvas duck was used for press cloths.

The procedure finally adopted was as follows: the filtering surfaces were given a precoat of 0.15 lb. coarse filter aid per square foot of filtering surface; 1% of the filter aid was mixed with the extract and the mixture then passed from a blow-case through the press under 40 to 50 lb. air pressure. The extract was kept hot by means of a steam jacket in the press. To the filtered extract was added 0.5% of activated powdered charcoal and the mixture stirred for one-half hour. The press was then cleaned and given a precoat similar to the first but using a finer filter aid. One per cent of the filter aid was also added to the extract which was then passed through the press as before. The filtrate was dried to yield solid preparations for test purposes.

All odour and taste were removed from all types of samples except fresh moss that had undergone some bacterial decomposition before reaching the laboratory. Most of the colour was also removed, but only the extract of leached, bleached samples could be called colourless. The extracts were progressively less coloured in this order: unbleached, leached unbleached, bleached, and leached bleached. It is realized that the figures given for concentrations of extract and quantities of filter aid and charcoal will not necessarily be suitable for industrial use.

Drying

Excessive heat applied to dry moss or solid extract produced hydrolysis. Nevertheless it was found that almost any method of drying could yield a product capable of forming good jellies. Since, as shown in Table I, the extracts contained only 0.23 to 0.47% solids, drying presented some difficulty. However, extracts were dried successfully on a laboratory model double-drum drier, an experimental spray drier, an experimental tunnel drier, and by concentrating the extract, allowing it to set, and drying small blocks of the resultant jelly with a fan at room temperature.

Extracts were fed to the drum drier from an overhead tank through tubes that ran the width of the drums. The drums were heated by steam at 50 lb. pressure. Since the knives could cut off the dry material only when it had attained a certain thickness on the drum, the material from dilute extracts remained on the drums longer than that from more concentrated extracts, and charring sometimes took place. Extracts preconcentrated to about 3% solids were dried successfully, although they were too viscous to be fed to the drums from the tubes described above. The dried product consisted of curled strips of solid and on grinding in a Christie mill yielded a powder of light brown colour.

Spray-drying produced the most attractive looking solid—a light, pure white powder, but this product was difficult to redissolve.

Extracts were tunnel-dried in galvanized sheet-iron trays with $\frac{3}{4}$ in. sides. A layer $\frac{1}{2}$ in. deep dried in four hours at 93° to 107° C., giving thin translucent sheets of solid that were slightly charred in spots. The ground product was similar in appearance to drum-dried solid.

Preconcentration of material for drying at room temperature required heating of the extract by steam at 100° C. in a jacketed open kettle for about four hours. The extract was constantly stirred mechanically and a stream of air was passed over its surface. As the extract was concentrated, some solid dried on the side of the kettle, and was collected (kettle-dried). The extract after preconcentration was allowed to set, cut into blocks about 2 in. × 2 in. × 1 in. and dried by a fan at room temperature for about 36 hr. Case-hardening always occurred. The ground product was similar in appearance to drum-dried and tunnel-dried material.

A method of preparing a pure gelose from Irish moss by precipitation with ethanol has been described (1). Owing to the large quantities of ethanol necessary to produce small amounts of dried gelose, this method is more suited to laboratory than to industrial use. Dry solid was prepared by precipitation with ethanol and also with acetone. The extract was concentrated to about 2 to 3%, divided into two equal portions, one portion poured with stirring into three times its volume of 95% ethanol and the other into a similar volume of acetone. The precipitate was removed as it formed, surplus liquid removed by squeezing, and the resulting material placed in sufficient fresh ethanol (95%) or acetone to cover it. After stand-

ing overnight the precipitates were removed from the cover-liquor, dried for 24 hr. at room temperature in slowly moving air, and then for two to three hours in an air-oven at 75° C. Approximately equal weights of solid were yielded by the two precipitants. The dried material was ground in a mortar and was then intermediate in appearance between spray-dried and drum-dried solids. Its ease of solution was intermediate also, indicating that with finer division of particles the dried extract was lighter in colour but more difficult to dissolve.

TABLE II

ANALYSES OF SAMPLES OF IRISH MOSS AND OF DRIED EXTRACTS PREPARED FROM IRISH MOSS BY VARIOUS METHODS

Type and treatment of moss	Method*	Moisture, %	Moisture-free basis		
			Ash, %	Chloride, %	Nitrogen, %
(a) Samples of Irish moss					
Unbleached moss					
Ground	—	8.0	29.7	1.37	1.20
Leached	—	10.4	21.8	0.21	1.63
Bleached moss					
Ground	—	9.6	22.9	0.14	1.00
Leached	—	11.1	19.2	0.06	0.93
(b) Dried extracts prepared from Irish moss					
Unbleached moss					
Whole	1. Drum	7.0	26.0	0.13	0.27
	3. Tunnel	2.6	30.0	3.72	0.27
Ground	2. Drum	5.1	37.8	6.72	0.27
	3. Tunnel	4.1	31.8	3.72	0.26
	4. Ethanol	11.2	24.6	0.06	0.20
	5. Acetone	11.2	27.5	0.06	0.22
Leached	2. Drum	9.3	23.5	0.08	0.08
	3. Tunnel	7.8	25.2	0.05	0.14
	4. Ethanol	6.8	19.8	0.08	0.11
	5. Acetone	6.6	21.1	0.06	0.11
Bleached moss					
Whole	1. Drum	7.8	25.0	0.09	0.15
	3. Tunnel	7.3	27.2	0.07	0.21
Ground	2. Drum	8.8	29.0	0.28	0.17
	3. Tunnel	9.5	29.7	0.29	0.16
	4. Ethanol	5.1	22.2	0.05	0.14
	5. Acetone	4.1	23.8	0.05	0.14
Leached	2. Drum	11.4	21.5	0.07	0.08
	3. Tunnel	7.1	23.4	0.02	0.14
	4. Ethanol	7.7	19.7	0.04	0.09
	5. Acetone	7.4	20.8	0.02	0.09

**Processes are described in text.*

The five methods of preparation used are listed in Table II. In Method 1 the air-dry moss, as received, was weighed into a 30-mesh screen, soaked in cold water for 10 min., and the filtered extract from this preparation drum-dried as described. The 10 min. soaking was omitted in the remaining

methods, the filtered extract being drum-dried in Method 2, tunnel-dried in Method 3, concentrated and precipitated with ethanol in Method 4, and concentrated and precipitated with acetone in Method 5.

Examination of Dried Extract

One gram samples were dried overnight in an air-oven at 98° C. to determine moisture and subsequently were ashed in an electric muffle furnace for 24 hr. at 550° C. Chlorides in the ash were determined by the Mohr method. Nitrogen determinations were made on 1 gm. samples by the Kjeldahl method. The results are shown in Table II.

TABLE III

EFFECTS ON JELLY STRENGTH OF ADDING VARIOUS SALTS TO 1% AGAR AND 2% GELS OF IRISH MOSS

Description of sample	Salt added, %	Second jelly strength, gm. Hg.	Appearance of final jelly
2% agar	None	41.6	Opaque
1% agar	None	8.2	Fairly opaque
	Sodium chloride, 0.1	—	Sample lost
	Calcium chloride, 0.1	3.3	Fairly opaque
	Ferric chloride, 0.1	0	Hydrolysed
Unbleached moss, leached, drum-dried	None	3.6	Clear
	Sodium chloride, 0.1	0	Viscous liquid
	Calcium chloride, 0.1	0	
	Ferric chloride, 0.1	0	Hydrolysed
Unbleached moss, ground, acetone precipitated	None	8.3	Slightly opaque
Unbleached moss, ground, ethanol precipitated	None	0	Clear
	Ash* ≈ 0.5	45.9	Opaque
Mixture of various extracts, No. 1	None	0	Slightly opaque
	Sea salt ≈ 0.4	4.7	
	Magnesium chloride, 0.1	0	
	Magnesium sulphate, 0.1	0	
	Potassium bromide, 0.1	5.2	Opaque
	Calcium sulphate, 0.1	0	Slightly opaque
	Calcium carbonate, 0.1	0	Opaque
	Potassium sulphate, 0.1	11.0	
Mixture of various extracts, No. 2	None	2.8	Slightly opaque
	Potassium sulphate, 0.1	13.2	
	Potassium carbonate, 0.1	20.3	Opaque, white ppt.
	Rochelle salt, 0.01	3.7	Slightly opaque
	.1	5.4	
	Potassium chloride, 0.01	4.2	
	.025	5.9	
	.05	8.4	
	.1	14.1	
	.2	33.7	
Bleached moss, ground, drum-dried	Potassium chloride, 0.2	62.9	Clear, ppt.
	.3	77.0	
	.4	95.8	
	.5	104.8	

* *Ash from preceding sample.*

Examination of Jellies

Since one of the criteria of the usefulness of any preparation was its ability to form a firm jelly, 2% solutions of each were prepared and the comparative strengths of the jellies determined. The instrument used for determining jelly strengths was constructed in these laboratories and has been described elsewhere (4).

Jellies were prepared as follows: 2 gm. of dried extract was weighed into 7-oz. flat sanitary cans, 100 ml. of boiling, distilled water was added, and the mixture stirred on a steam-bath for two minutes. The cans were then closed, retorted for 10 min. at 121° C. and cooled with running water. Jellies made with distilled water (Table IV) were not cooled after retorting; consequently

TABLE IV

STRENGTH OF JELLIES FROM 2% IRISH MOSS GELOSE, WITH AND WITHOUT ADDED POTASSIUM CHLORIDE

Type of moss used and method of drying extracts	With distilled water			With addition of 0.2% potassium chloride		
	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly
Fresh, wet moss						
Whole, drum-dried	28.9	29.3	Fairly opaque	31.8	57.4	Fairly opaque
Fresh, dried moss						
Whole, drum-dried	39.2	32.7	Opaque	34.5	54.8	Fairly opaque
Unbleached moss						
Whole, air-dried	17.4	22.1	Slightly opaque	25.6	39.4	Slightly opaque
drum-dried	9.5	9.4	Clear	24.2	51.0	Clear, dark ppt.
tunnel-dried	22.7	28.7	Fairly opaque	45.0	48.8	Opaque
Ground, kettle-dried	12.6	0	Clear, ppt.	—	—	—
drum-dried	19.4	22.2	Clear	31.5	42.1	Clear, dark
tunnel-dried	16.7	7.7	Slightly opaque	28.9	40.4	Fairly opaque
ethanol precipitated	11.9	0	Clear	36.7	52.0	Slightly opaque
acetone precipitated	11.5	5.8	Clear	36.8	55.7	Slightly opaque
Leached, kettle-dried	6.3	0	Opaque	—	—	—
drum-dried	3.6	0	Slightly opaque	22.1	42.6	Opaque
tunnel-dried	4.0	0	Opaque	45.4	41.9	Opaque
ethanol precipitated	5.9	0	Opaque	43.0	78.6	Fairly opaque
acetone precipitated	6.4	0	Opaque	32.1	73.0	Fairly opaque
Bleached moss						
Whole, air-dried	12.2	5.1	Clear	9.6	41.8	Slightly opaque
drum-dried	2.6	0	Clear	32.2	52.4	Slightly opaque
tunnel-dried	6.7	0	Slightly opaque	47.1	38.4	Slightly opaque
Ground, kettle-dried	6.4	0	Slightly opaque	45.7	52.9	Slightly opaque
drum-dried	7.4	0	Clear	53.3	62.9	Clear
tunnel-dried	7.9	0	Opaque	45.9	52.3	Opaque
ethanol precipitated	8.6	0	Clear	38.5	54.1	Slightly opaque
acetone precipitated	9.6	0	Slightly opaque	33.6	59.8	Slightly opaque
Leached, kettle-dried	5.4	0	Opaque	42.0	79.8	Opaque
drum-dried	4.0	0	Slightly opaque	82.7	81.6	Fairly opaque
tunnel-dried	6.8	0	Slightly opaque	28.8	27.6	Opaque
ethanol precipitated	13.1	0	Slightly opaque	67.2	91.4	Fairly opaque
acetone precipitated	22.6	0	Slightly opaque	77.9	90.0	Fairly opaque

they received more severe heating treatment than other preparations. The 10 min. retorting was in some instances insufficient to dissolve the dried extract completely, as evidenced by lumps of more concentrated mixture throughout the gel. This accounts for such discrepancies as greater jelly strengths for the longer retorting period (Table IV). The measure described in Table IV as first jelly strength was taken after the gels from the 10 min. retorting had stood overnight to come to room temperature (about 24° C.). After this measurement was made the cans were heated at 100° C. for five minutes and the contents transferred to new cans which were closed and retorted for one hour at 121° C. The second jelly strength measure was then made on these gels after standing overnight.

Addition of Potassium Chloride

Results in Table IV indicated that preparations from unbleached moss formed stronger jellies than those from bleached moss. It was also observed that leaching—in fact even the 10 min. soaking process—removed something from the extract that would otherwise have contributed to jelly strength. The fact that this substance was removed by the 10 min. soaking pointed to its being very soluble in water, suggesting a salt. Strengthening effects of alkali and alkaline earth salts on Irish moss jellies have been reported (3).

Various amounts of sodium, calcium, and ferric chlorides were added during the preparation of certain extracts. The only noticeable effect was the hydrolysis produced by ferric chloride (Table III). Additions of ash from preparations giving good jellies to preparations giving poor jellies resulted in jellies stronger than those yielded by the materials from which the ash was taken.

Sea water was used instead of distilled water in one preparation and the resulting jelly was stronger. A series of jellies was then made up with the addition of each of the various salts occurring in sea water (Table III). Potassium bromide was substituted for magnesium bromide, which was not on hand at the time. The results showed that only the potassium salts gave jellies of increased strength, and that jelly strength increased approximately in proportion to the amount of potassium added. This was particularly true of the jelly containing potassium carbonate which, however, contained considerable white precipitate, probably calcium carbonate. The jelly strength fell off slightly with Rochelle salt, potassium sulphate, and potassium chloride, in that order. However, potassium chloride was chosen as most economical with respect to its potassium content per unit weight, and jellies were made from all preparations with addition of this salt.

Table III shows that over the range of concentrations used, jelly strength increased in a straight line relationship with addition of potassium chloride. It is obvious that all added potassium is not taken up into the gelose structure. A potassium salt of the gelose prepared by dialysis has been reported to contain 12% potassium (1), but even 0.5% potassium chloride in these materials represents approximately 13% potassium with respect to gelose.

The effect of adding potassium chloride to preparations of the gelose was generally to equalize the strengths of resulting jellies. The jellies of highest strengths were those made from the preparations that were purest as shown by ash content; i.e. those made by precipitation with ethanol and acetone. The difference in jelly strength between leached bleached drum-dried and leached bleached tunnel-dried preparations may be accounted for on the basis of hydrolysis due to excessive heating in drying. This factor while not noticeable in the remaining data, may have had some effect.

While gelose from Irish moss appeared to go into solution at a lower temperature than agar, at a higher temperature agar went into solution more readily. During the stirring on the steam-bath, prior to closing the cans, part of the Irish moss gelose dissolved but the rest caked into small lumps throughout the liquid. Agar, however, in this stage, did not go into solution but was evenly dispersed throughout the liquid. After 10 min. retorting, agar had gone entirely into solution but lumps of undissolved Irish moss gelose still persisted. For this reason, comparisons between agar and Irish moss first jelly strengths (Table V) are not valid. Since both agar and Irish moss were completely dissolved at the end of the one hour retorting period,

TABLE V

STRENGTH OF JELLIES FROM AGAR AND IRISH MOSS GELOSE OF VARYING CONCENTRATION, WITH AND WITHOUT ADDED POTASSIUM CHLORIDE

Material tested	Concentration of gelose, %	With distilled water			With addition of 0.2% potassium chloride		
		First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly	First jelly strength, gm. Hg	Second jelly strength, gm. Hg	Appearance of final jelly
Agar	0.5	4.4	3.2	Slightly opaque			
	1.0	10.9	6.9	Fairly opaque			
	1.5	23.7	15.9	Opaque			
	2.0	41.0	27.6	Opaque	41.6	32.2	Opaque
	2.5	56.2	45.7	Opaque			
	3.0	79.3	63.1	Opaque			
Unbleached whole moss extract, air-dried at room temperature	0.5	0	0	Clear	5.1	4.5	Clear
	1.0	3.8	3.6	Clear	13.0	11.1	Clear
	1.5	7.0	7.8	Clear	22.8	19.5	Slightly opaque
	2.0	12.1	15.9	Clear	30.2	31.5	Slightly opaque
	2.5	22.4	26.8	Slightly opaque	41.8	49.6	Quite opaque
	3.0	24.1	38.0	Slightly opaque	42.7	59.9	Opaque

the second jelly strengths are comparable. Table V shows a series of Irish moss gelose, with and without added potassium chloride, and an agar series, at concentrations of from 0.5 to 3%. Although agar jellies were definitely stronger than those from equivalent concentrations of Irish moss when no potassium chloride was added, addition of this salt produced jellies from Irish moss that were stronger, in the main, than those from agar.

Hydrogen ion concentrations in jellies from Irish moss over a range from pH 8 to pH 6 apparently had no effect, but there appeared to be a gradual dropping off in strength below pH 6 (Table VI).

TABLE VI

EFFECT OF VARYING pH ON JELLY STRENGTH OF 2% GELOSE FROM UNBLEACHED WHOLE MOSS EXTRACT, AIR-DRIED AT ROOM TEMPERATURE

pH	Second jelly strength, gm. Hg	Appearance of final jelly
7.93	20.8	Slightly opaque
7.13	19.8	
6.13	20.0	
5.06	12.5	
4.15	4.0	

Taste Panel Tests

Since the purpose of the study was to investigate the usefulness of Irish moss gelose as a gelling agent with canned meat, an experimental pack of chicken was put up in 7-oz. cans. The chickens used were Grades *A* and *B* Milk Fed birds that had been in storage at −40° C. for over a year and had suffered some desiccation. An open precook of one hour was used after which the meat was removed from the bones and pooled. Ten cans were packed with 3½ oz. of meat, half light and half dark, taken at random from the whole supply. The broth was divided, salt and 2% agar were added to one-half and salt and 2% Irish moss gelose to the other. Three and one-half ounce portions of each of these broths were added to five cans containing meat. The cans were than closed and retorted for 45 min. at 121° C.

A panel of 24 tasters was asked to score samples of the product, with particular attention to the jelly, on a taste scale ranging from 10 for excellent to 0 for inedible. Under these conditions, a preference for agar of 0.75 score unit was shown by the tasters but this was below the level of significance ($t = 1.72$). It would therefore appear that Irish moss gelose has distinct possibilities as a substitute for agar. The work is continuing.

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THE IDENTIFICATION OF WIREWORMS OF ECONOMIC IMPORTANCE IN CANADA[1]*

By Robert Glen[2], Kenneth M. King[3], and A. P. Arnason[4]

Abstract

Over 30 different wireworm types are separated in the key presented. Their ecology and economics are discussed briefly and reference is given to the most important literature on biology, morphology, and control. Although descriptive morphological details are omitted, the separating characters given are derived from extensive detailed studies.

Perhaps in no field of economic entomology has progress been more retarded by lack of recognition of the species involved than in that dealing with wireworms. Although wireworm investigators have recognized the species with which they were working, other entomologists all too often have seemed to regard wireworms as constituting only a single economic form. In reports and textbooks especially, the subject has been treated very inadequately, reference usually being made simply to "wireworms". This has created the false impression that all wireworms are alike and that control measures are universally the same. As a result many data on habits, distribution, and control are of little value because of their generality.

The Preparation of Simple Field Keys

The primary objective of this paper is to provide basic information from which field men throughout Canada can prepare simple keys for the identification of the wireworm pests in their localities. The preparation of such keys is not difficult. A beginning can be made from the data here presented, to be supported ultimately by a local reference collection of reliably identified wireworms.

As a first step, a list of the pest species now known to occur in a particular province may be prepared by consulting Page 374. From the discussions in the text, these species may be further grouped on the basis of common habitats, viz., muck soil, parkland, open prairie country, etc. Examination of the illustrations and text will reveal the structural differences of the wireworms found in the same habitat. These gross differences of habitat and structure may then be integrated into a preliminary field key. The great majority of species that occur together in the same habitat are so distinct morphologically that a hand lens suffices for their identification, once the separating features are known.

* *An abstract of the main ideas embodied in this paper was prepared in 1938 (Reference 21).*

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An alternative or supplementary method is to develop the key coincident with the collecting of material. As soon as two different species are obtained, a simple key may be made for their separation. With further additions to the collection, the key should be expanded and amended until it includes all reliably named local forms.

The local key should not be merely a fraction of the "master key" here presented. In a key to all of the economic wireworms in Canada, it is necessary to make use, at times, of minute and relatively difficult characters. However, most practical field keys need include only gross structural differences. The identification of the larvae of *Ludius aeripennis destructor* and *Cryptohypnus nocturnus*, which occur together in western grain fields, serves as an example. To run these larvae through the master key would entail examination of many different structures, whereas reliable separation is possible by using a hand lens to examine the ninth abdominal segment (Figs. 45, 46 vs. 57, 53).

Regardless of how the local key is derived, a reliably named reference collection should be built up as soon as possible. The suggested procedure is to forward all unfamiliar wireworms to specialists for identification. The Saskatoon Laboratory is now in a position to offer assistance, both in making identifications and in supplying specimens of the common pest species.

Important Identifying Characters

In identifying wireworms the final decision must be based largely upon morphology, although information on habitat and habits has proved repeatedly to be of great assistance. Facility in using structural characters, as presented in the master key, depends primarily upon the following: (*a*) a working knowledge of the general morphology of elaterid larvae; (*b*) the reliability of the various taxonomic characters used; and (*c*) the common structural differences between young and mature larvae of the same species. These are discussed in the paragraphs that follow.

Although elaterid larvae are quite diverse in form (Figs. 1 to 11) they can be readily distinguished from larvae of other families occurring with them in farm land by the following combination of characters: three pairs of thoracic legs, well-developed, subequal in size, five-segmented (counting the terminal claw as a segment); labrum absent or fused with clypeus and anterior margin of frons into a rigid nasale (*n*, Fig. 12; Figs. 15 to 19); frontoclypeal area usually lyre-shaped (*fcl*, Fig. 12); maxillae and labium elongate, and fused into a single unit (*vmth*, Fig. 13); body straight, with nine abdominal segments visible dorsally, the ninth with or without paired terminal processes (= urogomphi); the 10th abdominal segment bears the anus and lies ventrad to the ninth and may or may not be armed by sclerotized tubercles or hooks; spiracles biforous.

False wireworms (Tenebrionidae), mainly of the genus *Eleodes*, bear superficial resemblance to some of the true wireworms of the subfamily Elaterinae. However, separation is relatively simple since false wireworms possess a well-

developed true labrum (*lbr*, Fig. 14), a Y-shaped frontal suture (*fs*, Fig. 14), more conspicuous antennae (*ant*, Fig. 14), an upturned tip and marginal spine-like setae on the ninth abdominal segment (Figs. 35, 41), and, in many species, enlarged prothoracic legs.

As far as possible, the most dependable characters have been selected for the key. Nevertheless, these are subject to some individual variation much of which is caused by "erosion"—a term applied to changes that occur during an instar as a result of feeding or movement through the soil. Structures that are subject to erosion are best observed on recently moulted specimens, since their taxonomic value is progressively reduced as the larva approaches the end of each instar. It is recommended that workers become familiar with the more important diagnostic structures and their relative dependability, from the following discussion.

The ninth abdominal segment (*9*, Figs. 1, 2, 7) is the most important structure in the identification of wireworms to genus and species. If lacking a median caudal notch: the tip of the segment may be flattened (Figs. 30, 31), smoothly rounded (Fig. 38), bluntly pointed (Figs. 33, 34, 40), or sharp (Figs. 32, 36, 37); the tergum may bear "eye spots" (*es*, Fig. 33) or whorls of preapical setiferous tubercles (*tub*, Figs. 36, 37, 40) or lack these; the sternum may be large (*st*, Figs. 34, 36, 47) or small. If possessing a median caudal notch: the notch, relative to the size of the segment, may be large (*cn*, Figs. 43, 45, 49) or small (*cn*, Figs. 58, 62); the urogomphi divided (*ur*, Figs. 44, 45, 49) or undivided (Figs. 42, 43), short and thick (Figs. 51, 53, 55) or relatively long and slender (Figs. 52, 54), with urogomphal prongs of various shapes; the dorsum of the segment may bear setae in the central area (Figs. 45, 50, 55) or lack such setae (Figs. 49, 57), and the lateral margins may be smooth (Fig. 64) or have protuberances that are blunt (*to*, Figs. 57, 62) or prominent, sharp and tooth-like (*to*, Figs. 47, 54). These characters are highly dependable. Erosion may dull and shorten the urogomphal prongs and some of the tooth-like protuberances, but the general character of the segment usually remains.

The nasale (*n*, Fig. 12) is a very useful structure, although usually requiring microscopic examination. It may be absent, or present as several teeth (Fig. 15) or as one tooth terminating in a single point (Fig. 16) or triple points (Figs. 17, 18, 19). It is subject to considerable erosion, but in the great majority of larvae the general type of nasale can be recognized even when quite worn. The nasale loses much of its diagnostic value when it is of a type having very small lateral denticles, which are commonly worn off.

Mandibles (*md*, Fig. 12) provide good separating characters, visible when fully opened. They may lack teeth on the inner aspect (Fig. 20), or possess one tooth, the "retinaculum" (*ret*, Fig. 22), or have teeth in addition to the retinaculum (Figs. 21, 23). Erosion may dull and shorten the points, but only minute denticles and ridges are likely to be completely worn away.

Prominent sculpturing, such as impressions (*im*, Figs. 26, 29, 30) and deep pits (Fig. 32), is an excellent supplementary character. The length of impressions and the density of pits frequently vary considerably between indi-

viduals of the same species and therefore are relatively unreliable characters for the separation of species in which the differences in sculpturing are slight. Sculpturing is less conspicuous on very immature larvae and on recently moulted specimens.

The presternum of the prothorax is a large triangular anterior area that may be composed of several pieces (*prst*, Fig. 27) or united into one sclerite (Fig. 24). The divided type is sometimes difficult to observe clearly in small larvae or in badly shrunken specimens. However, the character is of sufficient taxonomic importance to warrant practice in its use.

Eyes present or eyes absent is an excellent supplementary character. However, a binocular is required to observe the eyes and familiarity with their normal position is important. When present, the eye will be seen as a single, small, ovate, dark spot (under the integument) on the side of the head, situated a short distance posterior to, and in line with, the lower margin of the base of the antenna. One or more prominent setae usually occur just above the eye. The presence of eyes is more readily detected in fresh specimens. In prepupal or premoult larvae the eyes may be displaced. However, in species that have eyes the integument is not pigmented in the region normally occupied by the eye and the presence of a clear spot at the appropriate location may be taken as evidence of eyes being present.

Setae often vary markedly both in numbers and in arrangement. Contrasts, such as setae absent or setae present, are reliable differences, but as a rule setal characters should be supported by other evidence.

Size must be used with care, because the measure of length of a given specimen might vary considerably, depending upon the degree to which the specimen is distended. It is a useful supplementary character for unusually small or unusually large species.

Colour cannot be relied upon except between marked contrasts such as pale yellow and dark brown. Recently moulted specimens are always pale and for a time may lack characteristic colouring.

Biological characters. Little is known of the bionomics of most pest species and every opportunity should be taken to increase our knowledge. Information on habitat, life-history, and feeding habits often provides valuable clues to identification and very frequently is an essential complement to a relatively unreliable or obscure structural character. Sometimes all of the known larvae of a genus have similar habits. In such larvae, biological characters are of generic value. In other groups, the biology is diverse and difference in habit is specific. Some genera appear to be entirely predacious, and in many genera of economic importance there are several non-economic species living in wood or leaf litter. Such forms sometimes are encountered in fields of recently broken wooded land. Excellent reviews of the literature on biology and control of wireworms have been provided by Thomas (56, 57).

The characters used in the key were taken from well grown larvae. If younger specimens are examined slight differences may be expected. Young larvae usually differ from mature larvae of the same species by being some-

what paler in colour and by having fewer setae, tubercles, and denticles, and less conspicuous sculpturing. First instar larvae may differ markedly, especially in characters of nasale, caudal notch, and urogomphi. The number of larval instars of many elaterids varies so widely that it is not possible to determine the relative maturity of a given specimen. However, the key characters presented are known to hold for all larvae that are half grown or larger.

KEY TO WIREWORM PESTS IN CANADA†

1. Without a median caudal notch on ninth abdominal segment (Figs. 1 to 4)...(Subfamilies Cardiophorinae and Elaterinae)....2
   With a median caudal notch on ninth abdominal segment (*cn*, Figs. 5 to 11)...(Subfamilies Oestodinae and Pyrophorinae)....10
2. Abdomen with pseudo-segmentation (Fig. 1); larva extremely slender; mandible deeply cleft into ventral and dorsal branches. Widely distributed in sandy and light loam soils...(Subfamily Cardiophorinae)....**Cardiophorus* spp.
   Abdomen with normal segmentation (Figs. 2 to 11); mandible not of above type..(Subfamily Elaterinae)....3
3. Tip of ninth abdominal segment somewhat flattened and scalloped (Figs. 30, 31); usually with striate impressions anteriorly on dorsum of each abdominal segment (*im*, Figs. 4, 30); nasale of one single-pointed tooth (as in Fig. 16); eyes absent. Southern Manitoba and Eastern Canada....*Melanotus* spp.
   Tip of ninth abdominal segment not flattened (Figs. 34, 36)....4
4. Tip of ninth abdominal segment smoothly rounded (Fig. 38); sternum of ninth abdominal segment greatly reduced. Inhabiting decaying wood....**Parallelostethus* and related genera
   Tip of ninth abdominal segment pointed, either bluntly (Fig. 33) or sharply (Figs. 32, 37); sternum of ninth abdominal segment well developed (*st*, Figs. 34, 36)....5
5. With striate impressions and conspicuous pits on dorsum of abdominal segments (Fig. 32); nasale of one single-pointed tooth (as in Fig. 16). Widely distributed; in decaying wood....**Ampedus* spp.
   Without striate impressions or conspicuous pits on abdominal segments; nasale of one triple-pointed tooth (Fig. 19)....6
6. Two "eye spots" (conspicuous pigmented areas) near front of ninth abdominal segment (*es*, Fig. 33); mandible with tooth-like expansion of dorsal cutting edge (*to*, Fig. 23). Manitoba and Eastern Canada; generally in poorly drained fields and associated with grass....*Agriotes mancus*
   Without "eye spots" on ninth abdominal segment; mandible without expanded dorsal cutting edge (Fig. 22)....7
7. With small preapical setiferous tubercles‡ on ninth abdominal segment (*tub*, Figs. 36, 37, 40)....9
   Without such tubercles (Figs. 34, 39)....8
8. Ninth abdominal segment with blunt tip (Fig. 34). Widely distributed in park and forest areas....*Agriotes limosus*
   Ninth abdominal segment terminating in small nipple-like point (Fig. 39). Larva known only from Pemberton, B.C....Unidentified, probably *Agriotes* sp.
9. Ninth abdominal segment (Fig. 40) with blunt tip and one or two whorls of minute preapical tubercles. Southwestern Saskatchewan and southern Alberta; in open prairie country....*Agriotes criddlei*
   Ninth abdominal segment (Figs. 36, 37) with sharp tip and two or three whorls of small preapical tubercles. Widely distributed; usually in parkland or well-watered soils....*Dalopius* spp.

† *As an aid to the accurate identification of the economic species, characters are included for the separation of the common non-economic types encountered in soil and leaf litter. Species that have not been identified through rearing are shown by a question mark; non-economic forms are indicated by an asterisk.*

‡ *These tubercles may be very minute and easily overlooked by an inexperienced observer. They are merely slight elevations (usually more deeply pigmented) of the body wall around the bases of certain setae. Their presence is most readily detected by comparing the bases of setae that are situated well forward on the segment with those near the tip. Where the setal bases are similar throughout the segment, "setiferous tubercles" are lacking.*

10 (1). With two large, horny prongs issuing from dorsum of ninth abdominal segment (*dpr*, Fig. 5); nasale absent or greatly reduced...(Subfamily Oestodinae).....11
Without horny prongs in front of urogomphi (Figs. 6 to 11); nasale well-developed...(Subfamily Pyrophorinae).....12

11. Saskatchewan and Alberta; usually in alkaline situations.....*Oestodes puncticollis*
Eastern Canada and Manitoba.....*Oestodes tenuicollis*

12. Urogomphi undivided (Figs. 42, 43).....13
Urogomphi divided (Fig. 44), although sometimes outer prong is greatly reduced (*opr*, Figs. 45, 58, 64).....14

13. Urogomphi blunt, directed backward (Fig. 42); nasale of one single-pointed tooth (as in Fig. 16); eyes present. Widely distributed through northern forest; in leaf litter.....**Eanus decoratus*
Urogomphi pointed, incurving (Fig. 43); nasale of three short inconspicuous teeth; eyes absent. Prairie Provinces; in lighter soils of open prairie land.....*Hypnoidus dubius*

14. Without teeth on inner aspect of mandible (Fig. 20); head and prothorax reddish-brown, abdomen pale yellow and soft (see Fig. 6); nasale of three sharp teeth (Fig. 15); "anal armature" on 10th abdominal segment (*ar*, Fig. 47); ninth abdominal segment flattened, with prominent, sharp protuberances on lateral margins of dorsum (Figs. 44, 47). Ontario and Western Canada.....*Aeolus mellillus*
With teeth (usually only one tooth) on inner aspect of mandible (*ret*, Fig. 22); larva without contrasting colours on dorsum; nasale of one tooth, either single-pointed (Fig. 16) or triple-pointed (Figs. 17, 18, 19); without anal armature.....15

15. With large caudal notch on ninth abdominal segment (*cn*, Figs. 45, 49, 50, 54, 55, 56, 57), the posterior aperture of notch being wide.....16
With small caudal notch (*cn*, Figs. 58, 59, 60, 62, 63, 64) the posterior aperture of notch being narrow or entirely closed.....26

16. Outer prongs of urogomphi small, erect (*opr*, Figs. 45, 46); caudal notch U-shaped, with sides subparallel (*cn*, Fig. 45). Widely distributed; important crop pest on grass plains and parklands of Prairie Provinces.....*Cryptohypnus nocturnus*
Prongs of urogomphi subequal in length (Figs. 49, 50, 54, 55, 56, 57).....17

17. †With one small seta laterad to, but near each side of posterior part of frons (*ped*, Figs. 8, 25) and on anterior part of each body segment, as figured (*atm*, Figs. 8, 25); four setae on central dorsal area of ninth abdominal segment, the posterior setae being larger (Fig. 50); nasale of one triple-pointed tooth (lateral denticles sometimes worn off).....18
Without setae *ped* and *atm* (Figs. 7, 9, 10); with one of the following combinations of characters: (i) setae present on central dorsal area of ninth abdominal segment, nasale single pointed; or (ii) setae absent on above area, nasale single pointed; or (iii) setae absent on above area, nasale triple pointed.....19

18. Tip of outer prong of urogomphus curving backward (*opr*, Fig. 48); transverse branch of impressions on dorsum of first to eighth abdominal segments reaching approximately one-half distance to mid-dorsal line (as in Fig. 8). Eastern Saskatchewan, Manitoba, and Eastern Canada; usually in poorly drained soils.....*Cryptohypnus abbreviatus*
Tip of outer prong curving forward; transverse branch of impressions reaching to or nearly to mid-dorsal line on second to eighth abdominal segments (as in Fig. 7). In forested areas; in leaf litter.....**Ludius* spp.

19. One large triangular sclerite comprising presternum of prothorax (*prst*, Fig. 24); nasale of one triple-pointed tooth; sometimes with conspicuous punctures on dorsum of ninth abdominal segment (Fig. 49); other abdominal segments conspicuously sculptured with modified pits (Fig. 29).....20
More than one sclerite in presternum of prothorax (*prst*, Fig. 27); nasale of one single-pointed tooth; without conspicuous punctures on dorsum of abdominal segments (although sometimes with many fine, pin-point punctures).....21

20. Widely distributed; mainly in well-watered soils. Eyes absent.....*Hemicrepidius memnonius* and related spp.
Normally inhabiting decaying wood and leaf litter. Eyes present.....**Athous* spp.

† *The characters used in this couplet unavoidably are rather minute and therefore difficult. The critical feature here is the presence or absence of the setae ped and atm as shown in Fig. 25; the additional characters enumerated are merely supplementary. In a contracted larva, the setae atm may be covered by the intersegmental membranes. It should also be noted (as revealed in Couplet 18) that Cryptohypnus abbreviatus is the only species of economic importance that falls in the first half of Couplet 17. If the specimen under examination does not agree with Figs. 25, 48, and 50 or with the notes given in the discussion of the genus Cryptohypnus (p. 369) then the species falls in the second half of the couplet or is not known to be a pest species.*

21. Soil inhabiting. Tips of outer urogomphal prongs curving backward when not eroded (*opr*, Figs. 51, 52, 53); several spine-like setae on episterna of meso- and metathorax (Fig. 28) 22
Normally inhabiting decaying wood and leaf litter. Tips of outer urogomphal prongs either curving forward or bluntly rounded; without spine-like setae on episterna of meso- and metathorax ..... **Ludius* spp.

22. Urogomphal prongs like grappling hooks, dark and horny (Fig. 51); four or more fine setae on central dorsal area of ninth abdominal segment (Fig. 55). Saskatchewan and Alberta ..... *Ludius sexualis* (?)
Urogomphal prongs less curved, fleshier (Figs. 52, 53); none or four setae on central dorsal area of ninth abdominal segment 23

23. Four setae on central dorsal area of ninth abdominal segment (Fig. 56). Okanagan Valley, B.C.; in semiarid areas ..... *Ludius pruininus*
Without setae on this area 24

24. With sharp, horny protuberances on lateral margins of dorsum of ninth abdominal segment (*to*, Fig. 54); urogomphi relatively slender (Fig. 52; *ur*, Fig. 54) 25
With rounded protuberances on these margins (*to*, Fig. 57); urogomphi short and thick (Fig. 53; *ur*, Fig. 57) ..... *Ludius aeripennis*
Confined to the grasslands and adjacent park areas of the Prairie Provinces, including much of the Peace River section of Alberta and British Columbia; rarely exceeding 22 mm. in length when mature ..... subsp. *destructor*
Inhabiting the mountainous and forested regions to the west and north of the prairie grasslands, including the coastal area of British Columbia; usually exceeding 25 mm. in length when mature ..... subsp. *aeripennis*

25. Okanagan Valley, B.C., and southern Alberta ..... *Ludius glaucus*
Eastern Canada ..... **Ludius inflatus* (?)

26 (15). With two conical protuberances anteriorly on dorsum of ninth abdominal segment (*pro*, Fig. 64); outer prongs of urogomphi very small (*opr*, Fig. 64). Widely distributed through northern parklands ..... *Limonius pectoralis*
Without such protuberances 27

27. Extremely small outer prongs on urogomphi (*opr*, Fig. 58) 28
Prominent outer (upper) prongs on urogomphi (*opr*, Figs. 59, 60, 61, 62, 63) 29

28. In dry situations. Larvae known only from dry-farmed fields at Lethbridge, Alta. ..... Unidentified, probably *Limonius* sp. (Group 1)
In moist situations: muck soils or leaf litter. Widely distributed ..... *Limonius aeger*

29. More than one sclerite comprising presternum of prothorax (*prst*, Fig. 27); nasale of one single-pointed tooth (Fig. 16); eyes present; inner prongs of urogomphi flattened, outer prongs horn-shaped (Fig. 63). Widely distributed in parklands soil ... *Ludius kendalli*
One large triangular sclerite comprising presternum of prothorax (*prst*, Fig. 24); nasale of one triple-pointed tooth (lateral denticles sometimes worn off, see Couplet 31) 30

30. Soil inhabiting. Eyes absent 31
Normally inhabiting decaying wood and leaf litter. Eyes present ..... **Ludius* spp., **Athous* spp., and some related non-economic genera

31. With prominent tubercle-like protuberances along lateral margins of dorsum of ninth abdominal segment, sharply pointed when not eroded (*to*, Figs. 59, 60); lateral denticles of tip of nasale small (Fig. 17), frequently eroded giving appearance of single-pointed tooth 32
With comparatively inconspicuous wavy expansions in this location (*to*, Fig. 62); denticles of nasale subequal in size, rarely eroded completely 33

32. With five or more tooth-like protuberances along lateral margins of dorsum of ninth abdominal segment (*to*, Fig. 60); larvae usually less than 20 mm. in length. In parklands west of Great Lakes ..... *Ludius limoniiformis* (?)
With four protuberances in position described above (Fig. 59); larvae may exceed 30 mm. in length. Eastern Canada ..... *Ludius cylindriformis* (?)

33. British Columbia ..... *Limonius canus*
Eastern Canada (Figs. 26, 61, 62) ..... *Limonius ectypus* (?)
Prairie Provinces (Fig. 7) ..... *Limonius* sp. (Group 2)

Discussion by Taxonomic Groups

Elaterid larvae generally can be separated easily into one or other of the four subfamilies, Cardiophorinae, Oestodinae, Elaterinae, and Pyrophorinae, as recognized by Böving and Craighead (3).

Subfamily Cardiophorinae ("false-segmented" wireworms)

The *Cardiophorus* type (Fig. 1) is very different from other wireworms, having a thread-like body with nodulated pseudo-segmentation, forked mandibles, and two digitate anal processes. Such larvae are distributed widely over Canada, occurring in very sandy areas and to a lesser extent in light loam soils, and are probably chiefly predatory and non-economic. Larvae identified as *Cardiophorus* sp. have been reported (13, p. 28) as being destructive to corn in Illinois, but there are no Canadian records of their attack on plants. The *Cardiophorus* larva closely resembles that of *Horistonotus uhlerii* Horn, a species of considerable economic importance (1, 28, 55) in southeastern United States.

Subfamily Oestodinae

The *Oestodes* type (Fig. 5) is rare, but specimens have been recorded from Alberta to the Atlantic Coast. *Oestodes puncticollis* has been known to damage spring wheat and oats in alkaline parts of fields near Wadena, Sask., and to a lesser degree at Cadogan, Alta. *O. tenuicollis* occurs in Manitoba and Eastern Canada, but has not been associated with plant injury. Larvae of *tenuicollis* have not been reared, but the distribution of the species, based on adult collections, leaves little doubt as to the identity of the *Oestodes* larvae found in the East. The large dorsal prongs (*dpr*) on the ninth abdominal segment, and the greatly reduced nasale (sometimes absent) are characteristic of this genus. Further structural details are given by Hyslop (30, p. 251) and Böving and Craighead (3, Pl. 83).

Subfamily Elaterinae ("pointed-tail" or "round-tail" wireworms)

Larvae of the Elaterinae (Figs. 2, 3, 4) are characterized mainly by the posterior end which may be round or flat, blunt or sharp, but never notched in the middle. The body is cylindrical with greatly reduced pleural membranes. Generally speaking, the larvae thrive in moist or wet situations and possibly have a tendency to feed on decaying rather than on living vegetable matter, as observed by Ghilarov (14, pp. 634–635) for certain European species. In spite of this tendency several species become sufficiently abundant under Canadian farming and gardening practices to cause severe damage to cultivated crops. Many species are wood-inhabiting and of no economic importance; among these are all known larvae of *Ampedus* Dej. (= *Elater* auct.) and *Parallelostethus* O. Schw. and many species in other genera. The Canadian species of *Agriotella* Brown (5) are not known in the larval stage.

Genus *Agriotes* Eschscholtz

(Figs. 3, 22, 23, 33, 34, 39, 40)

Three Canadian species are known in the larval stage, *mancus*, *criddlei*, and *limosus*. In all of these the ninth abdominal segment (Figs. 33, 34, 40) terminates in a blunt point which distinguishes *Agriotes* from the closely related genus *Dalopius*.

A. mancus (Fig. 3) is pale yellow and frequently exceeds 20 mm. in length when full grown. It is readily distinguished by the two pigmented "eye spots" near the front of the ninth abdominal segment (*es*, Fig. 33), a character that is entirely lacking in *criddlei* and *limosus* (Fig. 34). *A. mancus* occurs in Manitoba and is widely distributed through Eastern Canada where it occurs mainly in poorly drained land, especially in muck soils and old meadows, and in cultivated fields where grasses have been grown in recent years. Moisture appears to be the principal factor governing its distribution since it has been taken in abundance from fields of very different soil types. Forbes (13, p. 34) states that the larva of *A. pubescens* Mels. is inseparable from that of *mancus*. Since adults of *pubescens* have been collected in Manitoba and in other parts of Canada, it is possible that in some sections the larvae of these species might be confused. However, all larvae of this type that have been reared to date at the Saskatoon Laboratory have proved to be *mancus*. These rearings include material from Manitoba, Ontario, Quebec, and New Brunswick. Much has been written on the ravages and control of this pest (22, 24, 25, 26, 45, 46, 47).

A. criddlei is reported (58) to be the smallest American species of *Agriotes*. The pale brown larva rarely exceeds 10 mm. in length. It is the only known *Agriotes* larva that possesses preapical setiferous tubercles on the ninth abdominal segment (Fig. 40). In this respect it resembles *Dalopius* larvae, but the latter have more prominent tubercles and a sharper point on the ninth abdominal segment. Larval specimens have been taken in southwestern Saskatchewan and in southern Alberta, including the irrigated districts. However, the species is known to occur as far east as Aweme, Man., as far west as Chilcotin, B.C., and as far north as Saskatoon, Sask. It is a larva of this type that is referred to by Strickland (51, p. 4) as ". . . . a small wireworm, which appears to be the larva of some species of *Agriotes*", and again by King (34, p. 705) as "*Agriotini*? . . . a rather small species of considerable importance in southwest Saskatchewan."

A. limosus is a pale brown larva measuring 18 or 19 mm. when full grown. It lacks both the "eye spots" of *mancus* and the "tubercles" of *criddlei*. It is a relatively rare species, but is widely distributed in the soil of park and forest areas and is believed to be of some economic importance in northern Saskatchewan. The larva has been described in detail (18).

An unidentified larva, which may belong to this genus, was collected by R. Glendenning of the Dominion Entomological Laboratory, Agassiz, B.C., on decaying turnips near the forest's edge of Pemberton, B.C. This larva resembles *A. limosus*, but has the tip of the ninth abdominal segment produced into a short, nipple-like point (Fig. 39).

Genus *Dalopius* Eschscholtz

(Figs. 2, 19, 36, 37)

The larvae of three species of *Dalopius*—*mirabilis*, *pallidus*, and *parvulus*—have been reared from cultivated fields. In all of these the ninth abdominal segment (Figs. 36, 37) terminates in a *sharp* point and bears three whorls (sometimes only two whorls in small specimens) of preapical setiferous tubercles. All are pale brown larvae rarely exceeding 15 mm. in length. Park and forest regions appear to be the typical habitat; the only specimens taken in open prairie districts have been collected from river flats and moist ravines. Specific larval characters have not been worked out.

D. pallidus occurs from Alberta to New Brunswick. It has been reported as a crop pest at Wetaskewin, Alta., and in the muck soils of Ontario and Quebec. In Saskatchewan, larvae have been taken from leaf litter on the banks of the South Saskatchewan River at Saskatoon. *D. parvulus* (Fig. 2) appears to be associated with grassy situations in the more open park sections of Saskatchewan and Manitoba, causing injury to grain crops when such land is put under cultivation. *D. mirabilis* is known (6, p. 95) from Alberta to Quebec and larvae have been taken from cultivated fields at a number of points in northern and eastern Saskatchewan and at Findlay, Man. Larvae were abundant under horse and cow manure in a native grass pasture at Mozart, Sask., in a definitely alkaline situation. Ten species of *Dalopius* are recorded (6, p. 35) from British Columbia, but the larvae are unknown.

Genus *Melanotus* Eschscholtz

(Figs. 4, 30, 31)

While specific characters are poorly known for *Melanotus* larvae, the genus is readily recognized by the ninth abdominal segment (Figs. 30, 31) which is flattened at the tip and usually more or less scalloped or lobed posteriorly. Excellent supplementary characters are enumerated in the key, Couplet 3. The larvae (Fig. 4) are somewhat darker than most wireworms, being brown or reddish brown with the head, thorax, and ninth abdominal segment slightly darker, the venter slightly paler. The more common species measure 20 to 30 mm., or more, when full grown. Larvae of this genus occur in abundance throughout Eastern Canada, and to some extent in the heavy clay soil of the Red River Valley in Manitoba and along some of the river courses as far west as the Saskatchewan–Manitoba boundary. The available records, while not extensive, suggest that in Canada the chief habitat is poorly drained land or soils with good moisture-holding capacity. Light infestations have been reported in muck soils. This is in general accord with most observations (11, 12, 27) on American species. However, in the State of Maine, *Melanotus* larvae are reported (24, p. 8; 26, p. 55) to be more numerous in light, relatively dry soil. On the basis of characters given by Forbes (13) the most abundant economic species in Canada appear to belong to the *communis* Gyll.—*fissilis*

Say group. In economic importance, *Melanotus* larvae rank second to *Agriotes mancus* throughout Eastern Canada. Damage has been reported (41, p. 530; 44, p. 3) to be worse after the ploughing of sod.

Subfamily Pyrophorinae ("forked-tail" or "notched-tail" wireworms)

The larvae of this subfamily (Figs. 6 to 11) are readily distinguished from those of the Elaterinae by the presence of a median notch at the posterior extremity. The body is more or less dorsoventrally flattened, usually with well-developed lateral membranes (*mem*, Fig. 26). Food and moisture requirements are diverse and some groups are highly predacious. At present, only six genera are known to be of economic importance in Canada, the larvae of most other genera apparently being confined to decaying wood and leaf mould. For the most part, the genera of this group are not as easily separated by larval characters as in the Elaterinae, combinations of characters being necessary for generic identification.

Genus *Aeolus* Eschscholtz (=*Drasterius* auct.)

(Figs. 6, 15, 20, 44, 47)

Only one species, *Aeolus mellillus*, is known in Canada. In the economic literature it is commonly referred to as *dorsalis* Say or *elegans* auct. Three subspecies have been recognized (4), but these are not separable in the larval stage. The larva (Fig. 6) is distinguished readily among the wireworm pests in Canada by the brown or reddish-brown head and prothorax which contrasts vividly with the pale yellow or whitish abdomen. The body is decidedly flat and specimens rarely exceed 15 mm. in length. The larva is notoriously predacious and its activity is noticeably greater than that of most wireworms. Damage by this wireworm frequently resembles the work of cutworms, the point of attack usually being near the soil surface and, in the case of young grain plants, the stems often are completely cut off. By contrast other wireworms bore into and shred the stems, but rarely sever them completely. Other diagnostic characters are given in the key, Couplet 14, and fairly detailed descriptions of the larva are given by Comstock and Slingerland (10) and by Jewett (31). Apparently the life cycle is completed normally in one year and development is by parthenogenesis in Canada and in some sections of the United States (33, 50). The species is found throughout Western Canada where it is a minor pest of cereals (34), particularly in southern districts and occurs as far east as Ontario where it has been reported (50) as severely injuring tobacco and truck crops in the southwestern peninsula. It inhabits native grassland and may be noticed as soon as such land is ploughed, but it also occurs commonly in old cultivated fields. Relatively dry situations seem to be preferred, although infestations occur in irrigated land and in soils of a wide range of texture. There are numerous records of plant injury by this pest in the United States (10, 13, 27, 31, 32, 49, 59). *Aeolus* is closely related to *Conoderus* Esch. (=*Monocrepidius* Esch.) and *Heteroderes* Latr., genera of economic significance in the United States.

Genus *Cryptohypnus* Eschscholtz

(Figs. 8, 25, 45, 46, 48, 50)

Accurate identification of the larvae of *Cryptohypnus* depends upon a combination of rather obscure characters. However, among the known wireworm pests of Canada, *Cryptohypnus* alone possesses the setae, labelled *ped* and *atm* in Figs. 8 and 25. Only two species, *nocturnus* and *abbreviatus*, are of economic significance in the Dominion. Both of these are pale yellow or bright yellow larvae with a large caudal notch, and reach 10 to 12 mm. in length when mature.

C. nocturnus (Fig. 8) is readily distinguished by the small, erect outer urogomphal prongs (*opr*, Figs. 45, 46) and by the U-shaped caudal notch which is not narrowed posteriorly. The larva has been described in detail (2). Adults of *nocturnus* have been taken from coast to coast in Canada, but records of crop injury are confined to Manitoba, Saskatchewan, Alberta, and the Peace River area of British Columbia. Over this range it ranks second to *Ludius aeripennis* as a pest of grain crops and surpasses it in importance in parts of the irrigated areas of Alberta. Although distributed throughout the open prairie and parklands it is relatively more abundant in areas of heavy soil, in grassy situations, and in fields that recently have been broken from sod (34). Control recommendations are discussed by King *et al.* (35, 36, 37).

In *C. abbreviatus* the urogomphal prongs (Figs. 48, 50) are subequal in length and the caudal notch is considerably narrowed posteriorly by the incurving tips of the inner prongs. A detailed description of the larva is given by Comstock and Slingerland (10, p. 270). This species is distributed throughout Eastern Canada, the most westerly record being Rhein, Sask. It inhabits soils of various textures, but appears to be more abundant in poorly drained land. In muck soils it is a minor pest.

Genus *Hemicrepidius* Germar (=*Asaphes* Kirby)

(Figs. 10, 29, 49)

Hemicrepidius larvae (Fig. 10) are bright yellowish-brown, and 20 to 30 mm. in length when mature. The large caudal notch (*cn*, Fig. 49), conspicuous sculpturing on the anterior half of the abdominal segments (Fig. 29), and the lack of eyes, when taken together distinguish the genus. Specific characters have not been worked out, but the larva of *H. hemipodus* Say (= *H. decoloratus* Say) has been described (10, 13). *H. memnonius* is the only Canadian pest species for which the larva has been identified by rearing. Although distributed throughout the Dominion, the larvae are rarely encountered in Western Canada and even in the East have never been reported as abundant. Moist situations such as watered gardens, muck soils, and low-lying land appear to be the favoured habitat.

Genus *Hypnoidus* Stephens

(Figs. 11, 21, 43)

Only one species, *Hypnoidus dubius*, has been encountered in cultivated fields in Canada. It is readily distinguished from other wireworm pests by the sharp, undivided incurving urogomphi (Fig. 43) and the large caudal notch. The larva (Fig. 11) rarely exceeds 10 mm. in length, has a soft, flattened body and is very pale yellow with slightly darker head and prothorax. It inhabits sandy loam soils in the open prairie sections of Saskatchewan, but is not definitely known to injure crops.

Genus *Limonius* Eschscholtz (=*Pheletes* Kiesenwetter)

(Figs. 7, 18, 24, 26, 58, 61, 62, 64)

Limonius larvae are bright yellow or yellowish-brown. The pest species can be distinguished from other Canadian wireworm pests by the following combination of characters: nasale of one triple-pointed tooth (as in Figs. 18, 19), small caudal notch (*cn*, Figs. 58, 62, 64), and well-rounded comparatively inconspicuous protuberances along the lateral margins of the dorsum of the ninth abdominal segment (Figs. 58, 62, 64). Although widely distributed in Canada the species are usually found in moist, and sometimes, alkaline situations. Similar observations on this genus are recorded by Lane (42, p. 530).

The larvae of the Canadian species of *Limonius* are poorly known; only *pectoralis*, *aeger*, *canus*, a species "near *ectypus* Say", and *consimilis* Walker (non-economic) have been identified through rearing. Although *Limonius* larvae can be recognized by the characters listed above, it facilitates identification of the pest species if these be considered as two distinct groups.

Group 1 includes *pectoralis*, *aeger*, and an unidentified larva. These species belong to different natural groups (17), but for convenience are considered here as a single unit. The larvae all have the outer urogomphal prongs reduced to mere tubercles (*opr*, Figs. 58, 64) and thus are distinguished from other wireworms having a small caudal notch. *L. pectoralis* measures up to 14 mm. in length and is readily distinguished by the two conical protuberances on the anterior part of the dorsum of the ninth abdominal segment (Fig. 64) and by the nasale in which the middle denticle is smallest (Fig. 18). It occurs widely in the Dominion, but only in the deep, black soils of the park belt of the Prairie Provinces and in the Peace River section of British Columbia is it known to be an important crop pest. In virgin land its typical habitat seems to be the grassy margins of snowberry (*Symphoricarpos*) thickets (34). Larvae believed to be *L. aeger* occur in the muck soils of southern Quebec where they are injurious to truck crops. However, in the Prairie Provinces the species has never been found in cultivated land although the larvae have been collected, and on several occasions reared to maturity, from moist leaf litter on the banks of the Saskatchewan River at Saskatoon. The unidentified species (Fig. 58) is known only from Lethbridge, Alta.,

and was taken by G. F. Manson of the Lethbridge Laboratory from a dry-farmed corn field where it caused considerable damage. Adults of *Limonius ursinus* Van Dyke have been collected in this locality.

Group 2 includes *canus*, *ectypus* (?), and a third species of uncertain identity. All are relatively large species, sometimes attaining 25 mm. in length. These larvae differ from *Group* 1 by having the outer urogomphal prongs about as long as the inner prongs (Figs. 61, 62) and may be distinguished from other pest species that have a small caudal notch by the combination of a triple-pointed nasale and relatively inconspicuous, well rounded protuberances along the lateral margins of the ninth abdominal segment (*to*, Fig. 62). Structurally, these species are very similar.

Canus and *ectypus* are almost indistinguishable. Both have long tergal impressions (*im*, Fig. 26) which, although varying considerably in length, nearly always clearly fall short of the mid-dorsal line. *L. canus* is a well-known pest in irrigated land and has been reported (48) as injuring truck and grain crops in the interior and coastal regions of British Columbia. The larva has been described in detail by Lanchester (38). *L. ectypus* (Figs. 26, 61, 62) is an eastern species and is known to attack tobacco and truck crops in southwestern Ontario.

The third species, while closely related to *canus* and *ectypus*, is distinct in both adult and larval stages. One adult has been reared, but specific identification has not yet proved possible. The larva is characterized by having tergal impressions that usually extend to the mid-dorsal line on the second to seventh abdominal segments (*im*, Fig. 7). Damage by this wireworm has been observed to cereals growing in low-lying, alkaline areas in open prairie and parkland margins in Manitoba, Saskatchewan, and Alberta. Usually only small spots in a field are affected. A few specimens have been found in irrigated land in Alberta.

Further information on the life-history, economic importance, and control of *Limonius* larvae is given by Graf (23) and by Lane (39, 41, 42, 43).

Genus *Ludius* Eschscholtz (= *Corymbites* Latreille)

(Figs. 9, 12, 13, 16, 17, 27, 28, 51 to 57, 59, 60, 63)

The larvae of seven species of *Ludius* have been taken from cultivated fields in Canada. These are too diverse in structure to be described as a single unit, and for practical purposes* are considered here as three groups.

Group 1 includes *sexualis*, *glaucus*, *inflatus*, *pruininus*, and *aeripennis*. These bright yellow larvae have a large caudal notch (*cn*, Figs. 54 to 57), the presternum of the prothorax divided into two or more sclerites (*prst*, Fig. 27), and lack the small setae that characterize *Cryptohypnus* (*ped*, *atm*, Figs. 8, 25). Specific separation depends entirely upon characters of the ninth abdominal segment.

* *In a study (17) of the known Ludius larvae of the world, 20 distinct "species groups" have been recognized. For convenience, Group 1 in this paper includes the economic species from several distinct, but closely related, Canadian groups.*

In *L. sexualis* (Fig. 55) this segment is almost square, with inconspicuous lateral protuberances (*to*), with four or more small hairs on the central dorsal area, and with the urogomphal prongs (Fig. 51) dark, horny, and converging much like grappling hooks. Specimens seldom exceed 16 mm. in length. Larvae have not been reared, but the identification is believed to be correct. The species is known from Alberta and Saskatchewan where it is associated in native situations with prairie grasses, especially in mixed grass and silverberry (*Elaeagnus*), and also to some extent in the grassy margins of snowberry (*Symphoricarpos*) thickets. It persists for many years after such areas are put under cultivation.

L. glaucus and *L. inflatus* are almost indistinguishable structurally and must be identified on the basis of distribution. These have relatively slender urogomphi (Figs. 52, 54), prominent tooth-like lateral projections (*to*), and no setae on the central dorsal area of the ninth abdominal segment. The largest specimens examined measured 18.5 mm. in length. Adults of *glaucus* have been taken (8) from the Okanagan Valley, B.C., and from southern Alberta. Larvae are known to injure grain crops in southwestern Alberta and along the foothills as far north as Calgary. Under the name "*inflatus* Say", damage by larvae of *glaucus* is reported by Hyslop (27, 29) and by Lane (39) in the northwestern United States. The true *inflatus* inhabits Eastern North America (8) and as far as is known is of no economic significance.

L. pruininus Horn (= *L. noxius* Hyslop), the great basin wireworm, is characterized (Fig. 56) by the stout urogomphi and relatively fleshy urogomphal prongs, the rather prominent lateral protuberances (*to*), and the four setae on the central dorsal area of the ninth abdominal segment. The larvae may exceed 20 mm. in length. In Canada, this species has been reported from the Okanagan Valley, B.C. It is a well known dry-land pest in the northwestern United States (27, 39, 40, 42), where its distribution is limited to the area that normally receives not more than 15 in. of rainfall annually.

L. aeripennis (Figs. 53, 57) closely resembles *pruininus*, but has less prominent protuberances (*to*) and lacks setae on the central dorsal region of the ninth abdominal segment. The typical *aeripennis* may attain 28 mm. in length, but the subspecies *destructor* rarely exceeds 22 mm. *L. aeripennis destructor* (Fig. 9), the prairie grain wireworm, is the outstanding wireworm pest in Canada. It is present over the whole agricultural section of the West, including the Peace River Block of Alberta and British Columbia; and also in the northern Great Plains states. Being a typical dry-land subspecies it is more abundant and more injurious on the well drained light and medium soils of the open prairies, but is also a pest in irrigated sandy soil. Grains and grasses are its favourite food and fields planted to these for five or more years in succession, without an intervening year of summer-fallow, usually become severely infested. The morphology of the larva has been described (15, 16, 17), its economic importance, ecology, and biology discussed (34, 52, 53, 54), and control measures enumerated (19, 20, 35, 36, 37, 52).

In the forested and mountainous areas to the north and west of the grasslands, the subspecies *destructor* is replaced by the larger typical *aeripennis* (7), which is a pest of truck crops and cereals in some of the mountain valleys and on the coastal area of British Columbia. Both subspecies are present in at least part of the Peace River Block of Alberta and British Columbia.

Group 2 is represented in Canada by the single species *L. kendalli* (=*L. virens* of American authors). This yellow-brown larva may attain 28 mm. in length and differs from those in *Group* 1 by having a small caudal notch (*cn*, Fig. 63). The divided prothoracic presternum (*prst*, Fig. 27) and the single-pointed nasale (Fig. 16) separate it from *Limonius* larvae. The species is widely distributed over the Dominion in northern parkland and forest (9), but records of damage are limited to northern Saskatchewan where, in localized areas, it is a serious pest for three or four years following the breaking of willow scrub land.

Group 3 includes *L. cylindriformis* and *L. limoniiformis*. The larvae of these species are yellow or yellowish-brown and have a small caudal notch (*cn*, Figs. 59, 60), the presternum of the prothorax undivided (*prst*, Fig. 24), and lack eyes. In these characters they resemble the pest species of *Limonius* from which they are distinguished by the small but prominent tubercle-like protuberances along the lateral margins of the dorsum of the ninth abdominal segment (*to*, Figs. 59, 60). Both species seem to prefer relatively moist situations. The only larvae of *cylindriformis* available to the authors were taken from a garden at Springhill, N.B., and were received through the courtesy of the Fredericton Laboratory. Adults are recorded from Nova Scotia and throughout southern Quebec and Ontario. The species is reported (26) to be of some economic significance in the State of Maine. Larvae believed to be *L. limoniiformis* have been taken from cultivated fields in the parklands of Saskatchewan; the adults are known (58, pp. 396, 420) from a wide area west of the Great Lakes, extending into Alberta.

Summary of the Regional Distribution of Pest Species

In Eastern Canada, which for present purposes includes a part of eastern Manitoba, the chief wireworm pests belong to the subfamily Elaterinae. The genera *Agriotes*, *Melanotus*, and *Dalopius* are widely distributed over this region, *A. mancus* being the most important pest species. In local sections of the East, severe losses are caused by wireworms of the subfamily Pyrophorinae, as in southwestern Ontario where *Aeolus mellillus* and *Limonius ectypus* frequently are troublesome. *Ludius cylindriformis*, *Cryptohypnus abbreviatus*, *Hemicrepidius memnonius*, and *Limonius aeger* have been reported as injurious in scattered localities.

In the muck soils—which are being increasingly used for vegetable production—the wireworms most commonly encountered are *Agriotes mancus*, *Dalopius pallidus*, and *Limonius aeger*. Species occurring in smaller numbers include *Cryptohypnus abbreviatus*, *Melanotus* spp., and *Hemicrepidius memnonius*.

The transition between the typically eastern and the typically western pest species is found in southeastern Manitoba. In the Red River Valley wireworms are not a serious pest, but in this area and further east *Agriotes mancus* and *Melanotus* spp. occur, sometimes together with a few specimens of *Ludius aeripennis* and *Cryptohypnus nocturnus*.

In Western Canada, the chief economic species are of the subfamily Pyrophorinae, mainly of the genera *Ludius*, *Cryptohypnus*, and *Limonius*. Larvae of *Dalopius* spp. and *Agriotes criddlei* are the only representatives of the subfamily Elaterinae that rank as important crop pests in this part of the Dominion while damage by larvae of the subfamily Oestodinae is extremely rare. *Ludius* larvae are found throughout this region. *Ludius aeripennis*, especially its subspecies *destructor*, is of outstanding economic importance.

In the open prairie country of Alberta, Saskatchewan, and Manitoba, and in the Peace River area of Alberta and British Columbia, *L. aeripennis destructor* predominates with *Cryptohypnus nocturnus* second in importance. *Limonius* sp. (near *ectypus*), *Hypnoidus dubius*, and *Aeolus mellillus* inhabit the southern prairie areas, but usually are present in smaller numbers and only in local situations. *Agriotes criddlei* is the only member of the Elaterinae inhabiting cultivated fields in typical open prairie locations. In the irrigated sections of southern Alberta, *C. nocturnus* is the chief pest of truck crops. Severe infestations of *L. destructor* are common in irrigated sandy land, but infrequent in the heavier soils, especially in fields that have received three or more floodings annually for several years. Larvae of *Aeolus mellillus*, *Agriotes criddlei*, and *Limonius* spp. occur in small numbers over the irrigated sections.

In the parklands of the Prairie Provinces, *L. aeripennis* and *C. nocturnus* are rather widely distributed, but the following species also may be present and sometimes predominant: *Limonius pectoralis*, *Ludius kendalli*, *Dalopius* spp., *Ludius sexualis*, *Ludius limoniiformis*, and *Agriotes limosus*. Rarely, in well watered sites, a few specimens may be encountered of *Hemicrepidius memnonius* and *Cryptohypnus abbreviatus*, but these have never been taken in abundance in the West.

The wireworms of British Columbia have not been studied carefully. *Limonius canus*, *Ludius aeripennis*, *Ludius glaucus*, and *Ludius pruininus* are pests in the Okanagan Valley and probably in other parts of the interior. *Aeripennis* and *canus* also occur along the Pacific Coast where they commonly injure truck crops. *Canus* is found chiefly in irrigated land while the others are typical dry land species.

List of Pest Species and Illustrations

The list is alphabetical, first by genera, then by species in each genus. Under "Distribution" is shown the region where each pest species is known to be, or likely to be, of economic importance. Page numbers refer to the principal discussions in the text. All identifications are believed to be correct,

but where there has not been verification by actual rearing a question mark is inserted. Certain non-economic species, included in the key as an aid to identification, are marked with an asterisk.

Species	Distribution	Figures	Page
Aeolus mellillus Say	S.W. Ontario, Prairie Provinces; probably British Columbia	6, 15, 20, 44, 47	368
Agriotes			365
criddlei Van Dyke	S.W. Saskatchewan, S. Alberta; probably throughout Western Canada	40	365
limosus Le Conte	Saskatchewan; probably Manitoba and Alberta	22, 34	365
mancus Say	Eastern Canada; probably eastern Manitoba	3, 23, 33	365
(?) sp.	British Columbia	39	366
**Ampedus* spp.		32	365
**Athous* spp.			
**Cardiophorus* spp.		1	365
Cryptohypnus			369
abbreviatus Say	Eastern Canada, Manitoba, eastern Saskatchewan	25, 48, 50	369
nocturnus Eschscholtz	Western Canada	8, 45, 46	369
Dalopius			367
mirabilis Brown	Manitoba, Saskatchewan; probably from Quebec to Alberta		367
pallidus Brown	Quebec, Ontario, Alberta; probably from New Brunswick to Alberta		367
parvulus Brown	Manitoba, Saskatchewan	2, 19, 36, 37	367
spp.	Throughout Canada		367
**Eanus decoratus* Mannerheim		42	
Eleodes spp. (false wireworms)		14, 35, 41	359
Hemicrepidius			369
memnonius Herbst	Throughout Canada, except British Columbia	47	369
spp.	Throughout Canada	10, 29	369
Hypnoidus dubius Horn	Saskatchewan, probably Manitoba and Alberta	11, 21, 43	370
Limonius			370
aeger Le Conte	Quebec; probably Eastern Canada		370
canus Le Conte	British Columbia		371
ectypus Say (?)	Ontario; probably Quebec	24, 26, 61, 62	371
pectoralis Le Conte	Western Canada	18, 64	370
sp. (Group 1) possibly *ursinus* Van Dyke	Southern Alberta	58	370
sp. (Group 2)	Prairie Provinces	7	371

Species	Distribution	Figures	Page
Ludius			371
aeripennis aeripennis Kirby	Western Canada (northern forests and western mountains and coast)		372
aeripennis destructor Brown	Prairie Provinces and Peace River Block, B.C. (open prairie and park)	9, 12, 13, 27, 28, 53, 57	372
cylindriformis Herbst (?)	New Brunswick; probably Eastern Canada	59	373
glaucus Germar	Southern Alberta, interior British Columbia	52, 54	371
**inflatus* Say (?)			371
kendalli Kirby	Saskatchewan, probably Prairie Provinces	16, 63	373
limoniiformis Horn (?)	Saskatchewan, probably Prairie Provinces	17, 60	373
pruininus Horn	Interior British Columbia	56	371
sexualis Brown (?)	Saskatchewan; probably Prairie Provinces	51, 55	371
*spp.			
Melanotus spp.	Eastern Canada, Manitoba	4, 30, 31	367
Oestodes			
puncticollis Horn	Saskatchewan, Alberta		365
tenuicollis Randall	Eastern Canada, Manitoba	5	365
**Parallelostethus attenuatus* Say		38	365

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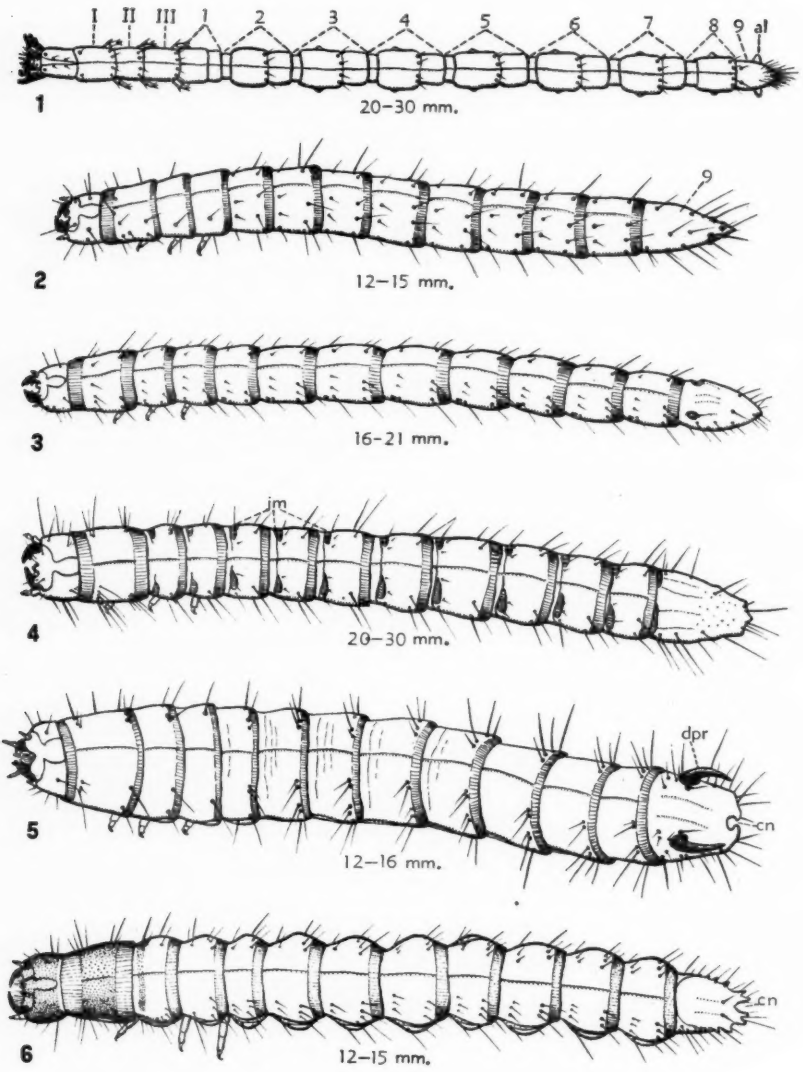
Abbreviations Used in Illustrations

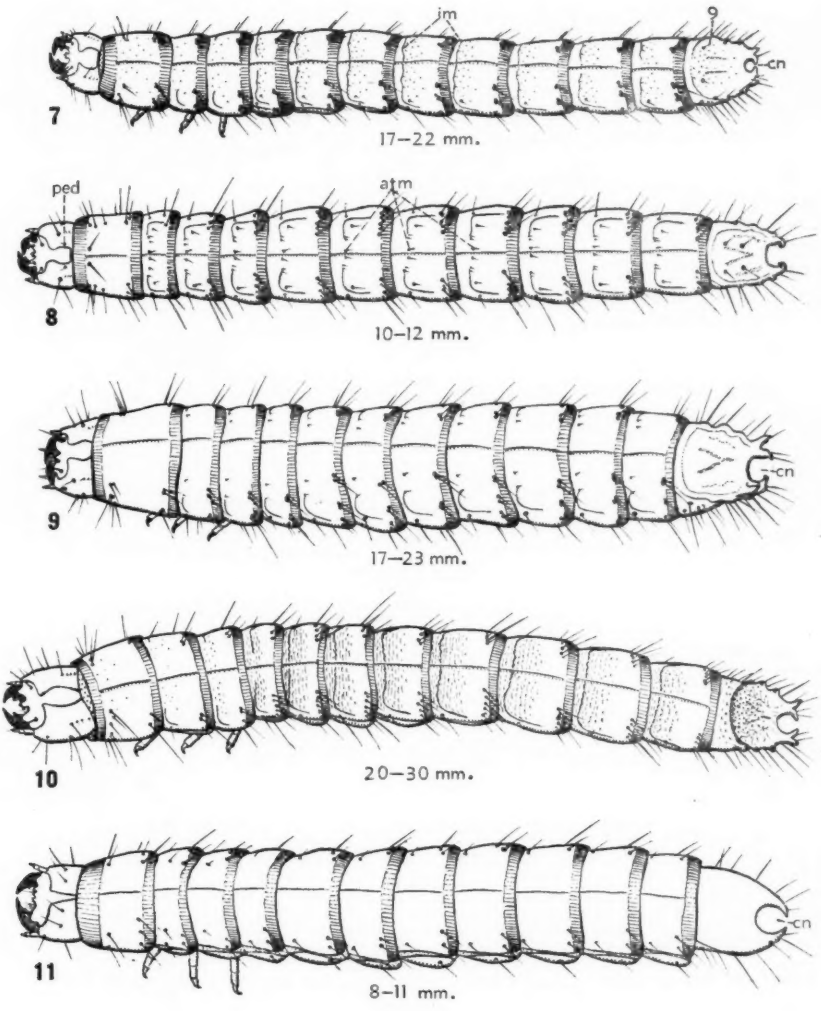
(See pp. 380-387)

Only the structures of primary taxonomic importance have been labelled. The terminology used was developed during recent detailed studies (17, 18).

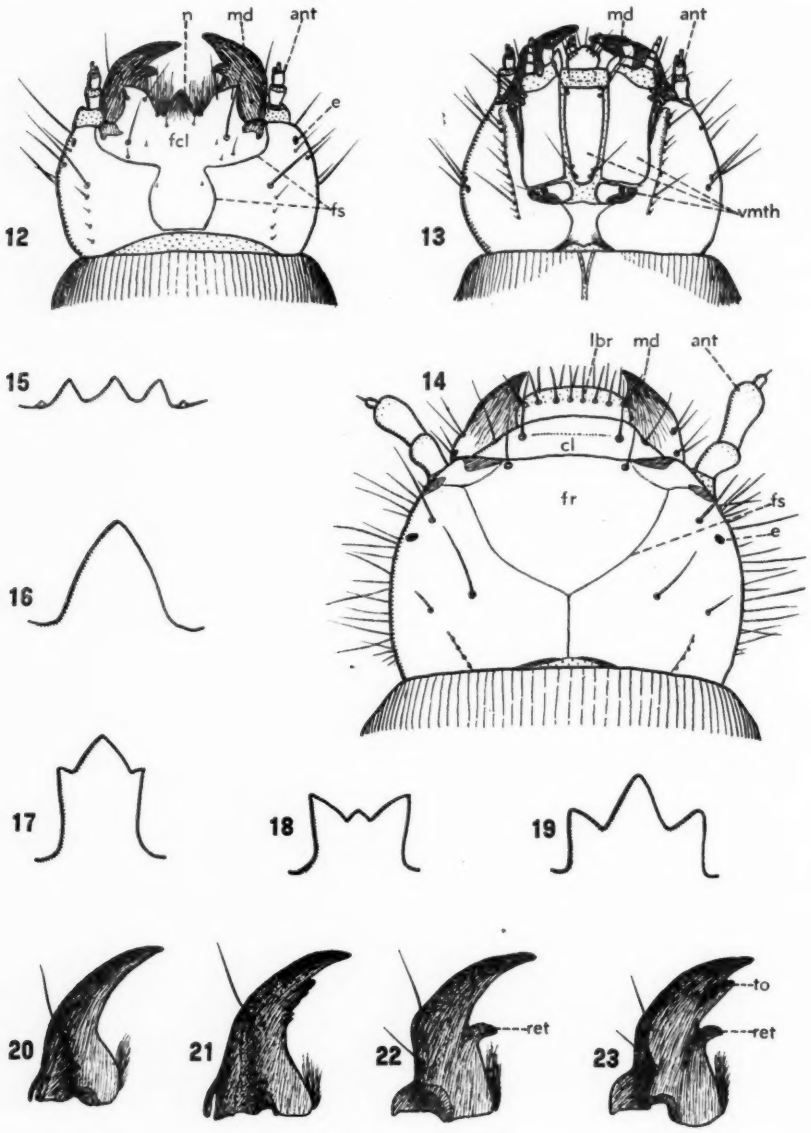
al, anal lobe
ant, antenna
ar, anal armature
atm, medial anterotergal seta
cl, clypeus (in "false" wireworms)
cn, caudal notch
cx, coxa
dpr, dorsal prongs
e, eye
epst, episternum
es, pigmented eye-like impressions or "eye spots" on ninth abdominal segment
fcl, frontoclypeal region
fr, frons (in "false" wireworms)
fs, frontal suture
im, impression
ipr, inner or lower prong of urogomphus
lbr, labrum (in "false" wireworms)
md, mandible
mem, membrane
n, nasale
opr, outer or upper prong of urogomphus
ped, dorsal posteroepicranial seta
pro, protuberance
prst, presternal area
ret, retinaculum
sp, spiracle
st, sternum
tg, tergum
to, tooth-like protuberance
tub, tubercle
ur, urogomphus (="cercus")
vmth, ventral mouth parts: the unit formed by the fusion of maxillae and labium
1 to 10, abdominal segments
I, *II*, *III*, thoracic segments



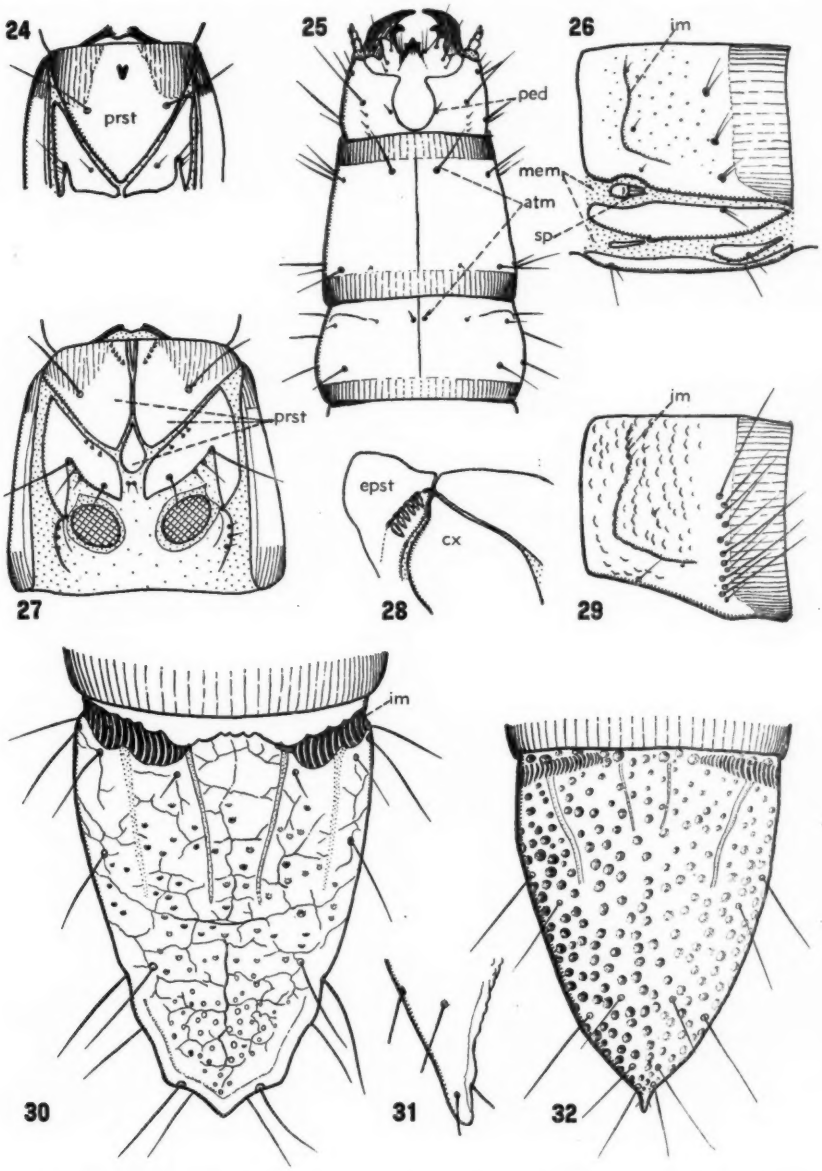
FIGS. 1 TO 6. *Types of wireworms (dorsal view). Length at maturity shown under each figure.* 1. *Cardiophorus sp.* 2. *Dalopius parvulus.* 3. *Agriotes mancus.* 4. *Melanotus sp.* 5. *Oestodes tenuicollis.* 6. *Aeolus mellillus.*



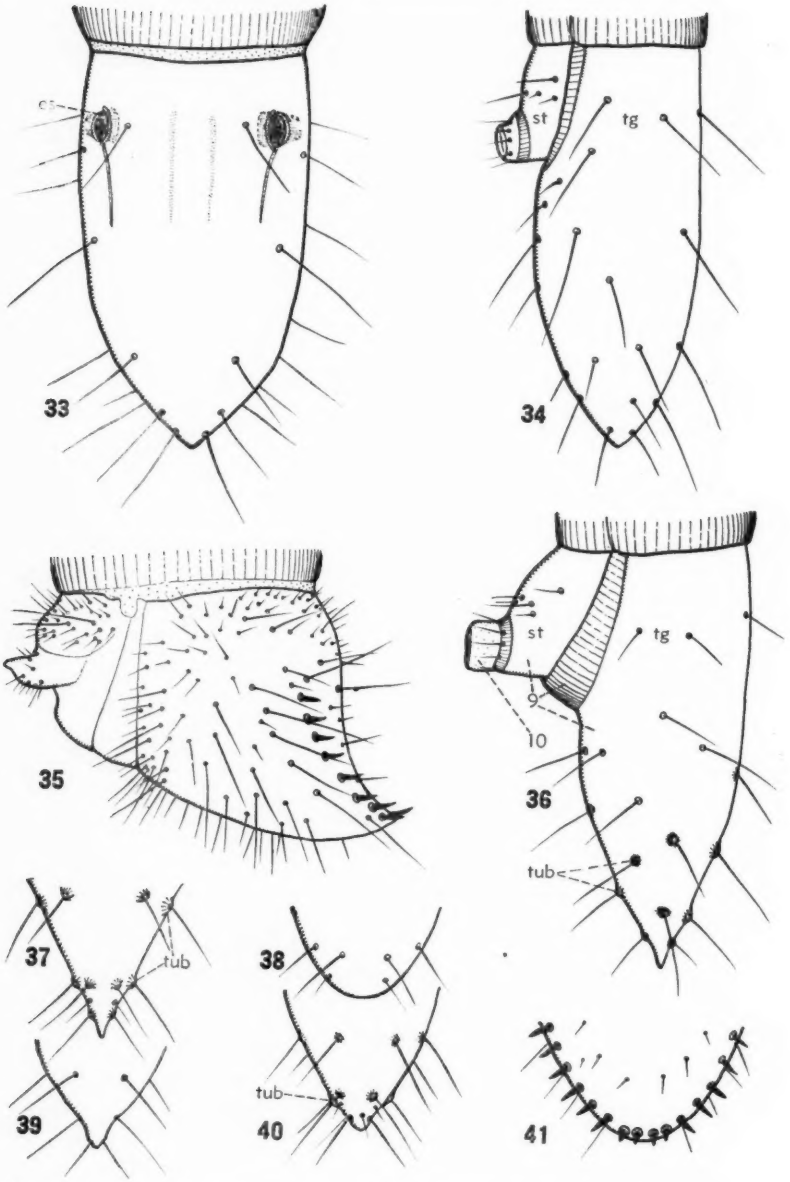
FIGS. 7 TO 11. *Types of wireworms (dorsal view). Length at maturity shown under each figure.* 7. *Limonius sp. (Group 2).* 8. *Cryptohypnus nocturnus.* 9. *Ludius aeripennis destructor.* 10. *Hemicrepidius sp.* 11. *Hypnoidus dubius.*



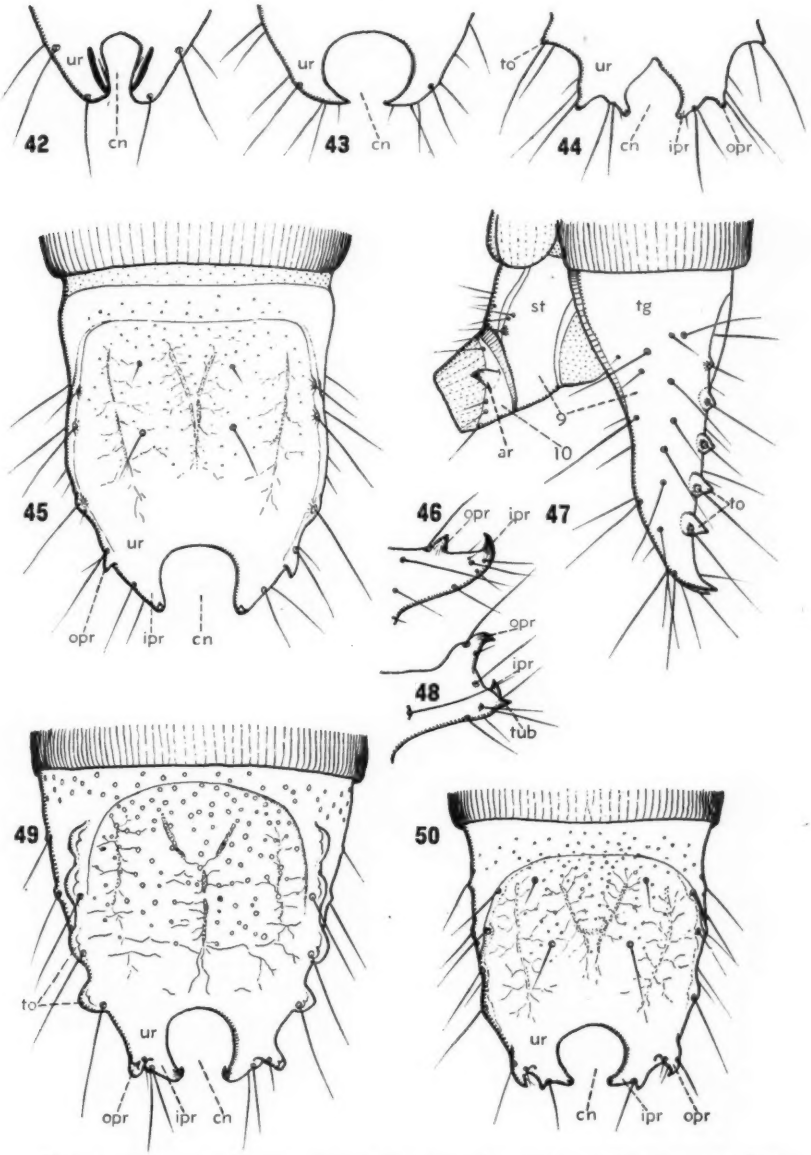
FIGS. 12 TO 23. 12. *Ludius aeripennis destructor, head, dorsal.* 13. *L. destructor, head, ventral.* 14. *Eleodes sp. (Tenebrionidae), head, dorsal.* 15. *Aeolus mellillus, nasale, dorsal.* 16. *Ludius kendalli, nasale, dorsal.* 17. *Ludius limoniiformis, nasale, dorsal.* 18. *Limonius pectoralis, nasale, dorsal.* 19. *Dalopius parvulus, nasale, dorsal.* 20. *Aeolus mellillus, left mandible, dorsal.* 21. *Hypnoidus dubius, left mandible, dorsal.* 22. *Agriotes limosus, left mandible, dorsal.* 23. *Agriotes mancus, left mandible, dorsal.*



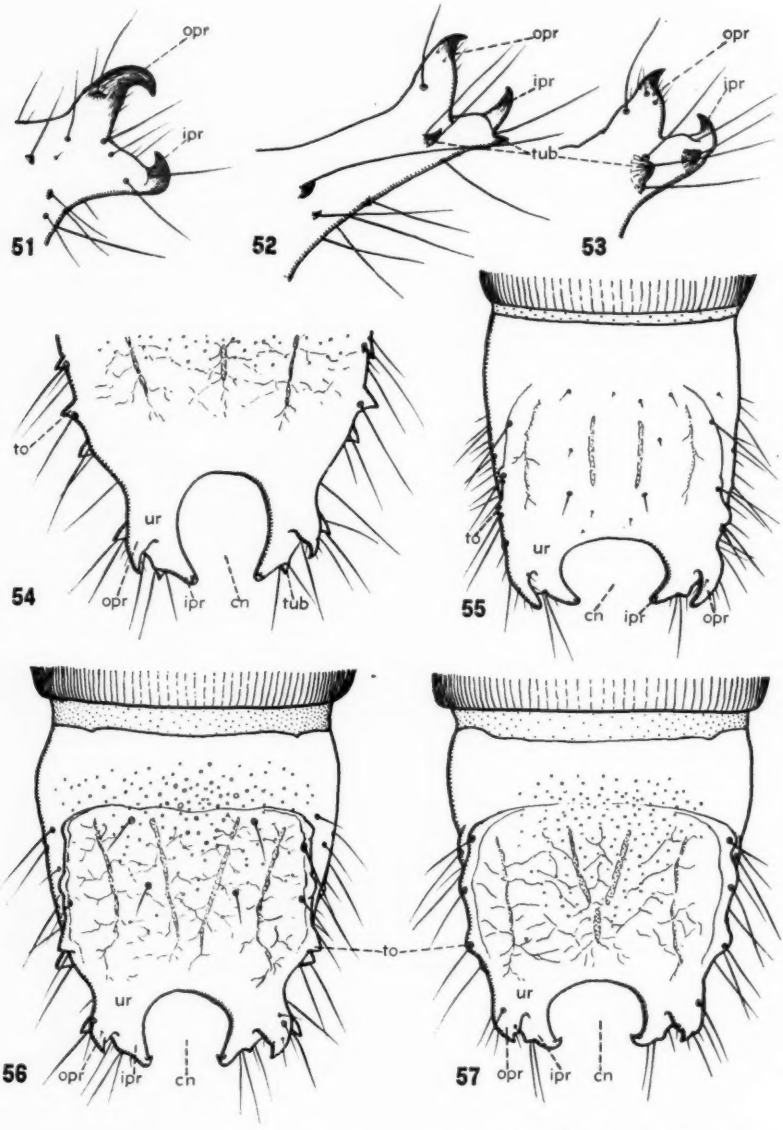
FIGS. 24 TO 32. 24. *Limonius ectypus (?), anterior part of prothorax, ventral.* 25. *Cryptohypnus abbreviatus, head, prothorax, and mesothorax, dorsal.* 26. *Limonius ectypus (?), fifth abdominal segment, dorsolateral.* 27. *Ludius aeripennis destructor, prothorax (legs removed), ventral.* 28. *L. destructor, episternum of mesothorax, lateral, to show spine-like setae.* 29. *Hemicrepidius sp., left half of dorsum of fourth abdominal segment, dorsolateral.* 30. *Melanotus sp., ninth abdominal segment, dorsal.* 31. *Melanotus sp., tip of ninth abdominal segment, lateral.* 32. *Ampedus sp., ninth abdominal segment, dorsal.*



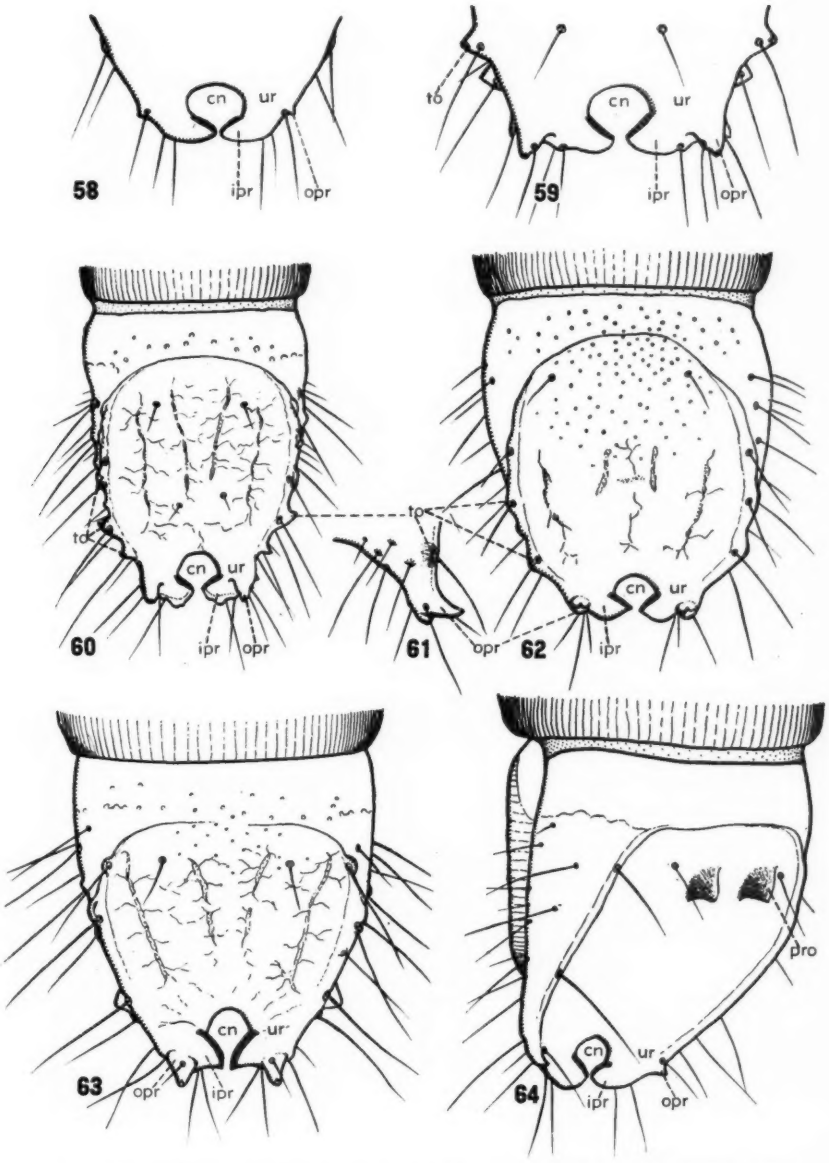
FIGS. 33 TO 41. 33. *Agriotes mancus, ninth abdominal segment, dorsal.* 34. *Agriotes limosus, ninth and 10th abdominal segments, lateral.* 35. *Eleodes sp. (Tenebrionidae), ninth and 10th abdominal segments, lateral.* 36. *Dalopius parvulus, ninth and 10th abdominal segments, lateral.* 37. *D. parvulus, tip of ninth abdominal segment, dorsal.* 38. *Parallelostethus attenuatus, tip of ninth abdominal segment, dorsal.* 39. *Agriotes (?) sp., tip of ninth abdominal segment, dorsal.* 40. *Agriotes criddlei, tip of ninth abdominal segment, dorsal.* 41. *Eleodes sp. (Tenebrionidae), tip of ninth abdominal segment, dorsal.*



FIGS. 42 TO 50. 42. *Eanus decoratus, tip of ninth abdominal segment, dorsal.* 43. *Hypnoidus dubius, tip of ninth abdominal segment, dorsal.* 44. *Aeolus mellillus, tip of ninth abdominal segment, dorsal.* 45. *Cryptohypnus nocturnus, ninth abdominal segment, dorsal.* 46. *C. nocturnus, tip of ninth abdominal segment, lateral.* 47. *Aeolus mellillus, ninth and 10th abdominal segments, lateral.* 48. *Cryptohypnus abbreviatus, tip of ninth abdominal segment, lateral.* 49. *Hemicrepidius memnonius, ninth abdominal segment, dorsal, drawn from larval exuvium of a reared specimen.* 50. *Cryptohypnus abbreviatus, ninth abdominal segment, dorsal.*



FIGS. 51 TO 57. 51. *Ludius sexualis (?), tip of ninth abdominal segment, lateral.* 52. *Ludius glaucus, tip of ninth abdominal segment, lateral.* 53. *Ludius aeripennis destructor, tip of ninth abdominal segment, lateral.* 54. *Ludius glaucus, posterior half of ninth abdominal segment, dorsal.* 55. *Ludius sexualis (?), ninth abdominal segment, dorsal.* 56. *Ludius pruininus, ninth abdominal segment, dorsal.* 57. *Ludius aeripennis destructor, ninth abdominal segment, dorsal.*



FIGS. 58 TO 64. 58. *Limonius sp. (Group 1), tip of ninth abdominal segment, dorsal.* 59. *Ludius cylindriformis (?), tip of ninth abdominal segment, dorsal.* 60. *Ludius limoniiformis (?), ninth abdominal segment, dorsal.* 61. *Limonius ectypus (?), tip of ninth abdominal segment, lateral.* 62. *L. ectypus (?), ninth abdominal segment, dorsal.* 63. *Ludius kendalli, ninth abdominal segment, dorsal.* 64. *Limonius pectoralis, ninth abdominal segment, dorsolateral.*

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